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Phytochemical Analysis and Bioactivities Studies of Fresh Leaves and Flowers from *C. roseus*, *L. multiflora* and *P. amarus*, Beninese Medicinal Plants Used Against Diabetes

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Abstract: Traditional herbal medicines make up a large part of drug use. This work addressed an area of public health, the fight against diabetes. The present study aimed to carry out qualitative and quantitative screening of the secondary metabolites of extracts from the leaves and flowers of plants such as *Catharanthus roseus*, *Lippia multiflora* and *Phyllanthus amarus*, and to evaluate the antioxidant activity in their aqueous and ethanolic extracts, their larval toxicity and then to predict the antidiabetic activity. The results showed that its plants have in their various extracts some secondary metabolites such as alkaloids, tannins, flavonoids, coumarins, terpenoids, phenolic compounds, steroids, etc responsible for several interesting pharmaceutical activities. These medicinal plants showed a higher quantity of polyphenols in the hydro-ethanolic extracts, with levels of 60.65±0.13; 44.40±1.43 and 67.11±0.39 mg.eq/g respectively for *C. roseus*, *L. multiflora* and *P. amarus* and also a higher antioxidant potential for *L. multiflora* and *P. amarus* than the standard Vitamin C, with IC_{50} equal to 0.53 and 0.52 mg/mL respectively. Extracts are not toxic against larvae. These results are an important indicator of the hypoglycemic and anti-diabetic activity and confirmed the potential for use of these plants by the population in the fight against diabetes.

Keywords: Phytochemical Analysis, Anti-Diabetic, Antioxidant Potential, Hydro-Ethanolic Extracts

1. Introduction

In the traditional treatment of diseases, medicinal plants, through their leaves and flowers play an essential role by displaying different biological activities and bioefficacity. Metabolic and pathologic disease related to Diabetes, one of the major degenerative chronic diseases in the world characterized by a disorder in the regulation of blood glucose levels [1, 2]. Various symptoms associated with diabetes are fatigue, frequent urination, blurred vision, mood changes, excessive thirst, etc. Diabetes is associated with various long-term complications affecting the heart, retina, kidney, liver and nervous system. Diabetes is a disorder of carbohydrate metabolism in which glucose molecules are not oxidized properly due to lack of insulin [3]. The accumulation of unused sugar molecules lead to their

appearance in the blood and urine. Despite the health risks that diabetes poses to humans, almost half of all diabetics are unaware of their condition, increasing the number of diabetics from year to year. However, among the continents, Africa remains one of the most affected by diabetes, with an estimated around 16 millions adults suffering from diabetes in sub-Saharan Africa, and 70% of them undiagnosed [4]. The lack of resources in the continent's underdeveloped countries, such as Benin, leads people to turn to plants as a great source of active ingredients for treatment. Traditional self-medication with medicinal plants is the mainstay of curative medicine for modest-income populations. This social class to prevent or treat illness uses many plants species with anti-diabetic activity [5, 6], with strong clinical action and get free from the annoying property like acidity, uncomforting taste, and allergy and kidney problems formed by artificial medicines [7, 8]. Although some of these plants are highly reputed in both traditional and modern medicine, much more work remains to be done in the field of scientific research to ensure total satisfaction for man without exposing his life. Apart from different synthetic drugs, a large number of herbal drugs have been used for the treatment of diabetic patients. In Benin medicinal ethnobotanical system, a large number of medicinal plants are used for the treatment of diabetes as three medicinal plants have been employed in the treatment of type II diabetes [9, 10]. Furthermore, the present study aims to carry out a phytcochemical characterization of potential bioelements [11] in the treatment of type II diabetes from medicinal plants. Indeed, normal components have forever been an attractive form for detecting remedies, and various natural medicines have been developed to care for diabetes, ethnopharmacological use; additionally, around 1200 kinds of herbs have been monitored for antidiabetic action [12, 13]. There has been increasing demand of herbal medicinal products with antidiabetic activity, low cost, and reduced side effects as foreword to their antioxidant activities in drug synthesis [14, 15].

The medicinal plants are found to be the most important source in primary health care system in the developing countries. The therapeutic activity of a plant depends upon the bioactive compounds present in those plant parts used in preparation of the herbal medicine. Plants contain a number of biologically active components referred to as secondary metabolites, which are biosynthesized during the normal metabolic processes of plants. Secondary metabolites are mainly responsible for the biological activities, which also promote health benefit effects. The medicinal properties of the plants lie in various chemical substances that also produce some physiologic actions on human bodies. The most important of these bioactive constituents are steroids, alkaloids, glycosides, flavonoids, terpenoids, carotenoids, tannins and phenolic compounds [16, 17]. A large number of purified constituents of these medicinal plants have shown beneficial therapeutic effects. Medicinal plants are found to be the richest bio-resources of traditional folk medicines. Natural medicines are gradually becoming a popular part of healthcare system worldwide [18, 19].

Three antidiabetic medicinal plants namely *Catharanthus roseus*, *Lippia multiflora* and *Phyllanthus amarus* have been selected for the present study. The present investigation was carried out to analyse the phytochemical constituents of *Catharanthus roseus*, *Lippia multiflora* and *Phyllanthus amarus* about their leaves and flowers. The present study was also aimed to characterize the functional groups present in crude ethanolic extract of these medicinal plant extracts, and to evaluate and predict the antidiabetic activity. More specifically, the aim is to carry out quantitative and qualitative screening of the secondary metabolites of extracts from the aerial parts of these medicinal plants, to assay and select the major compounds with antioxidant power; to highlight the antidiabetic virtue of the plants through evaluation of the anti-radicalar activity and larval toxicity of the selected extracts.

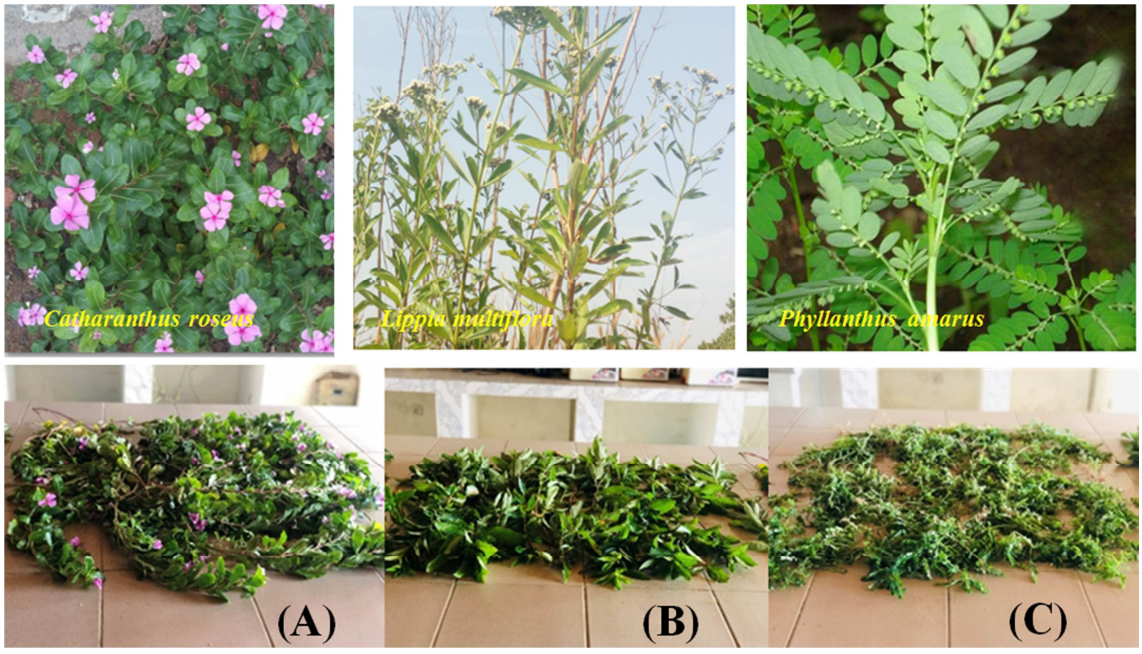

Figure 1. *Fresh leaves and flowers of C. roseus (A), L. multiflora (B), P. amarus (C) dried in the laboratory conditions.*

2. Material and Methods

2.1. Material

The vegetal material was the leaf and flower powders from three species which are *Catharanthus roseus*, *Lippia multiflora* and *Phyllanthus amarus*. They were collected in the department of Collines, more precisely in the commune of Dassa-Zoume in centre au Benin, cleaned and debris-free with clean water, then lab bench at a temperature of 22°C for two weeks.

2.2. Methods

2.2.1. Phytochemical Screening

To carry out the phytochemical screening of the various groups of compounds, we first collected 5 g of powder of each plant in various small bottles, which we infused for 30 minutes in 100 ml of boiling water. After filtration using a system consisting of a small cotton pad + funnel, the filtrate was collected in tubes with the addition of reagents for identification of the various phytochemical groups.

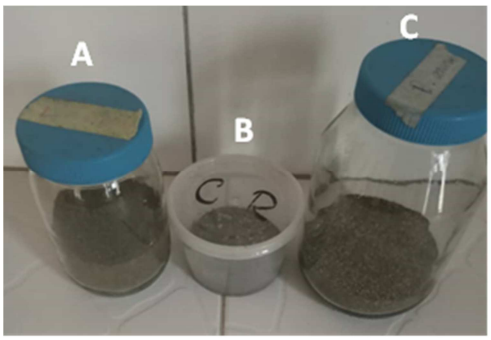

Figure 2. *Powders of L. multiflora (A), C. roseus (B), P. amarus (C).*

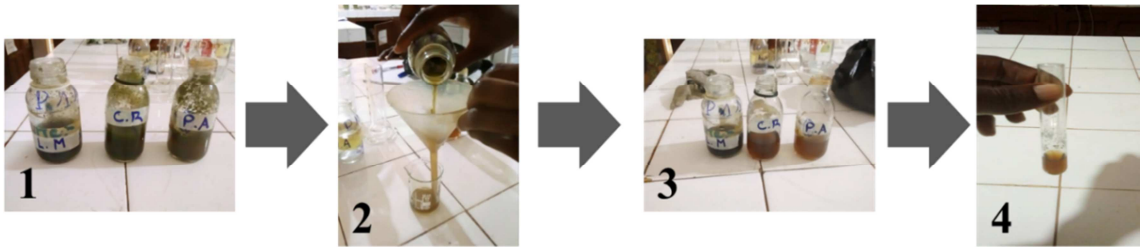

Figure 3. *Extraction process for phytochemical dosage of L. multiflora (A), C. roseus (B), P. amarus (C).*

Table 1. *Identification of secondary metabolites and reactions in plant extracts.*

Active Substances	Specific reagent and Reaction
Alkaloids	- Dragendorff (potassium iodobismuthate) → orange precipitate - Mayer (potassium iodomercurate) → yellowish precipitate
Tannins	- $FeCl_3$ → dark-blue coloring
Flavonoids	- Shinoda (cyanidine reaction) → orange-red coloring
Anthocyanins	- Red coloration in acid medium and purplish blue in alkaline medium
Leuco-anthocyanins	- Alcohol Cl (EtOH 50°/HCl_{cc} 2: 1) → cherry red coloring
Quinonic derivatives	- Bornträger (reaction between quinonic rings in a medium NH_4OH) → purplish-red color
Saponosides	- Determination of foam index (positive if IM > 100)
Steroids and Terpenes	- Liebermann-Burchard (Acetic anhydride-H_2SO_{4cc} 50: 1) → violet coloring - Kedde (dinitrobenzoic acid 1% in EtOH + NaOH 1N 1: 1) → purple-red color (cardenolides)
Cyanogenetic derivatives	- Guignard (Paper impregnated with picric acid) → brown coloring
Mucilages	- Study the viscosity of infused and decocted products

The phytochemical screening was based on the differential reactions (staining and precipitation) of the main groups of chemical compounds contained in plants according to the classic method widely and successfully used in the literature [20, 21]. This analysis includes the identification of secondary metabolites, the reactions of which are described as follows in Table 1.

2.2.2. Preparation of Crude Extracts

Total chemical principles were extracted from the powders of the various plant species using the maceration method, in accordance with their traditional use. To obtain the macerates, the mixture of 50 g of drug with 500 ml of solvent (water/ethanol in 40/60 v/v; distilled water) is left to stir continuously for 48 hours. The filtrate obtained is then filtered and concentrated to dryness using a rotavapor at 40°C. Extraction is repeated three times on the same quantity of 50 g powder.

Figure 4. *Extraction of active ingredients in the three plants.*

The various dry residues obtained are weighed and the yield is calculated according to the expression:

$$Rdt\ (\%) = \frac{Extract\ weight}{Powder\ weight}\ X\ 100 \quad (1)$$

In order to use the extracts for pharmacological and toxicological tests, it seems important to preserve the bioactive state of the extracted molecules [22]. We therefore stored them at low temperature (24°C).

2.2.3. Determination of Total Phenols

A sampling of 125 µL at 1 mg/ml is taken and dissolved in 625 µL of Folin-Ciocalteu reagent. After incubation for 5 min, 500 µL of sodium carbonate Na_2CO_3 at 75 mg/ml is added. The mixture is vortexes and incubated for 2 hours in the dark. Absorbance readings are taken using a Genova spectrophotometer at 760 nm [23]. Polyphenol concentrations are deduced from calibration ranges established with Gallic acid (0-10 mg/ml) and are expressed in mg Gallic acid equivalent (GA) per gram of dry extract [24, 25] according to the expression:

$$T\left(\frac{mgeqAG}{g}\right) = \frac{C.V_r}{V_p.C_p} \quad (2)$$

Where, T = Compounds content, C = Concentration obtained from calibration curve, V_r = Reaction volume, V_p = Volume of extract, Cp = Concentration of sample extract.

2.2.4. Determination of Flavonoids Content

Take 500 µL of $AlCl_3$ solution (2%) and add 500 µL of sample. Add 3 ml methanol and incubate for 10 min. Optical densities are read using a spectrophotometer at 415 nm against a blank consisting of 500 µL $AlCl_3$ and 3.5 ml methanol. Quercetin (Q) was used as a control, prepared at a concentration of 10mg/ml methanol [24]. Flavonoid contents are calculated from the regression line of the standard (Quercetin) and are expressed in mg Quercetin equivalent per gram of dry extract [26], according to the same expression in 2.2.1, by replacing Gallic acid (GA) with Quercetine (Q).

2.2.5. Determination of Condensed Tannins

Taking 500 µL of the extract, we added 1.5 ml of the vanillin solution (4%) prepared in methanol, 1.5 ml of concentrated hydrochloric acid and 2 ml of methanol. The vanillin solution is prepared by dissolving 4 g vanillin in 100 ml methanol, and the catechin solution is prepared from 20 mg catechin in 4 ml methanol [27]. The mixture is incubated for 15 min and the absorbance is read at 500 nm. The calibration line is established with catechin (0-500 µg/ml). Tannin contents are calculated from the regression line of the standard (catechin) and expressed in µg of catechin equivalent (CAT) per milligram of dry extract according to the same expression in 2.2.1, by replacing Quercetine (Q) with Catechin (CAT).

2.2.6. Determination of Antioxidant Activity

The DPPH• test measures the free radical scavenging capacity of pure molecules or plant extracts in a model system (organic solvent, room temperature). It measures the ability of an antioxidant (AH, usually phenolic compounds) to reduce the chemical radical DPPH• (2,2-diphenyl-1-picrylhydrazyl) by hydrogen transfer as radical donor at 517 nm (λmax DPPH•) [28, 29]. The initially violet DPPH• is transformed into the pale yellow DPPH-H.

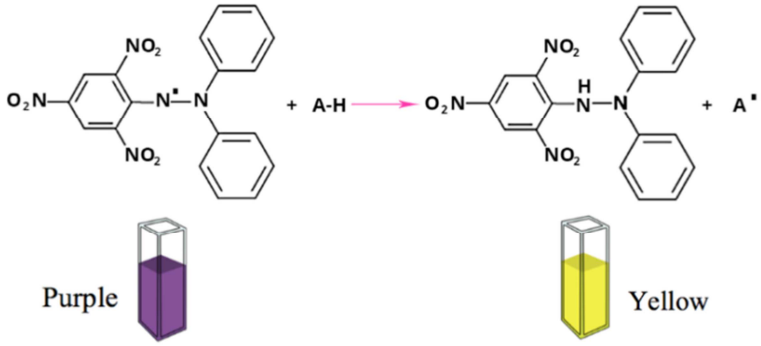

Figure 5. *Antioxidant activity reaction pathway by using DPPH• test.*

A stock solution of each sample is prepared at 1000 ppm by dissolving 1 mg of extract in 1 ml of methanol, and then diluted to one-tenth. 4 mg DPPH• is dissolved in 10 ml methanol to give a mass concentration of 0.4 mg/ml for the standard DPPH• solution. Next, 1.5 ml of the diluted extract solution is mixed with 3 ml of the DPPH• methanoic solution.

The free radical scavenging activity of the extract was determined using a calibration curve established with Ascorbic acid (0-10 mg/ml). The percentage of free radicals scavenged by DPPH• is calculated according to the following relationship:

$$\%DPPH^{\bullet} = \frac{Absorb\ blank(control) - Absorb\ sample(extract)}{Absorb\ blank\ (control)} \times 100 \quad (3)$$

Where $Absorb\ blank(control)$ is the absorbance of the control (reaction mixture excluding test compounds) and $Absorb\ sample\ (extract)$ is the absorbance of the test compounds. The IC50 value, which corresponds to the extract concentration that caused 50% DPPH• radical neutralization, is calculated from the graph of reduction percentages as a function of extract concentration. Each test is performed in duplicate. The optical density reading was taken against a blank prepared for each concentration at 517 nm after 15 min

of incubation in the dark at room temperature. A solution of a standard antioxidant represents the positive control, ascorbic acid, whose optical density was also measured under the same conditions as the samples. For each concentration, the test was repeated 3 times.

3. Results and Discussion

3.1. Extraction Yield

The two aqueous and hydro-ethanolic extracts of our plants were obtained from fine powders of the leaves and flowers of these plants, using two different extraction methods. The yield is calculated in relation to the total weight of the powder and is given in the following Table 2.

From this analysis, it was observed that the hydro-ethanol extractions of our different plants showed significantly higher yields than the aqueous extractions. The difference in yield between the two extracts is due to the extraction techniques used, which are totally different, and to the chemical composition, which differs from one extract to the other. The observation that hydro-ethanol extraction is higher than aqueous extraction could be justified by the mixed nature of this solvent, which enables both polar compounds to be bound by water and non-polar compounds by ethanol. Indeed, the extraction capacity of a solvent depends, on the one hand, on the affinity of this solvent towards phyto-molecules and, on the other hand, on the polarity of this solvent [30].

Table 2. *Plants extracts yields investigations in hydro-ethanolic (HE) and Aqueous (Aq).*

Samples Extracts	Mi (g)	Δm (g)		R		R (%)	
		HE	Aq	HE	Aq	HE	Aq
Catharanthus roseus	50	18,449	6,235	0,369	0,124	36,99±0.18	12,47±0.14
Lippia multiflora	50	10,830	4,893	0,216	0,097	21,66±0.13	9,78±0.06
Phyllanthus amarus	50	11,669	7,120	0,233	0,142	23,33±0.21	14,24±0.17

MI: Initial mass, Δm: Extract mass, R: yield.

3.2. Phytochemical Compounds

The results obtained after phytochemical screening are summarized in Table 3.

Table 3. *Preliminary phytochemical screening of crude extracts of C. roseus, L. multiflora and P. amarus.*

Chemical Groups				Plants		
				C. roseus	*L. multiflora*	*P. amarus*
Phenolic compounds	Tannins	Catechic tannins		+	-	+
		Gallic tannins		+	+	+
	Flavonoïdes	Flavones		+	+	+
		Leuco-anthocyanins		+	-	+
	Anthocyanins			+	+	-
	Anthracene derivatives	Free		-	-	-
		Combination	O-heteroside	-	-	+
			C-heteroside	-	-	-
			C. Reducers	-	-	+
	Coumarins			+	-	-
Nitrogen compounds	Alkaloids			+	+	+
	Steroids			+	-	-
Steroids and terpenoids	Saponosides			-	-	-
	Triterpenoides			-	-	-
Cardiotonic heteroside				-	-	-
Mucilage				-	-	-
Cyanogenetic derivatives				-	-	-
Quinonic derivatives				-	-	-

Legend: + = Presence, - = Absence

Phytochemical screening of the various plant powders revealed the presence of alkaloids, flavones and gall tannins in all three plants; catechic tannins and leuco-anthocyanins in *C. roseus* and *P. amarus*; coumarins and steroids only in *C. roseus* extract; and reducing compounds and O-heterosides in *P. amarus* extract. However, anthracene derivatives, saponosides, triterpenoids, cardiotonic heterosides, mucilages, cyanogenetic derivatives and quinone derivatives were absent from samples of all three plants.

What's more, the species studied do not contain toxic chemical groups (cardiotonic and cyanogenic derivatives), making them a priori safe for oral use. It is the phytochemical constituents that give a plant its medicinal properties [31]. Based on these results, the phenolics identified in the

phytochemical tests need to be assayed, and their yield determined.

3.3. Content of Some Pharmacological Groups

In order to characterize the extracts of the three plants, total polyphenols, flavonoids and tannins were determined, using the absorbance of the molecules, these families of compounds of therapeutic interest were quantified to justify the antioxidant activity recognized in *C. roseus*, *L. multiflora* and *P. amarus*. Absorbance readings were taken using a Biomax 770 nm spectrophotometer. The following absorbance figures show the calibration curves for the standards used to determine phenols, flavonoids and total tannins respectively. The calculated contents of total phenols, total flavonoids and total tannins are summarized in the tables below.

3.3.1. Total Phenol Content

Gallic acid was used as the standard. Levels were reported in milligram equivalents of Gallic acid per gram extract (mgeqAG/g) of plant material.

Furthermore, the results indicate a higher level of polyphenols in the hydro-ethanolic extracts of all three plants than in their aqueous extracts. Indeed, the aqueous (29.44 ± 1.17) and hydro-ethanolic (67.11 ± 0.39) extracts of *Phyllanthus amarus* have a higher polyphenol content than those of *Lippia multiflora* (27.04 ± 4.04 and 44.40 ± 1.43) and *Catharanthus roseus* (21.96 ± 6.79 and 60.65 ± 0.13).

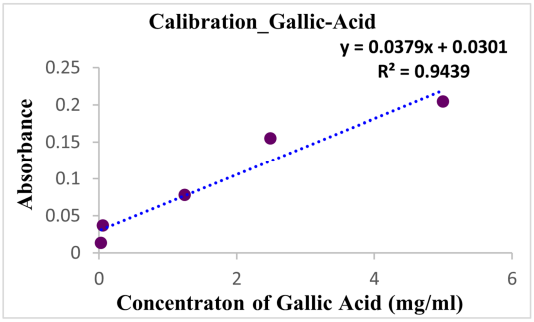

Figure 6. *Gallic acid calibration curve.*

Table 4. *Total polyphenol content of aqueous and hydro-ethanol plant extracts.*

Plants	Extracts	Calibration Equation	Concentrations	Contents (mg*eq*AG/g)
Catharanthus roseus	Aq.Extract	y = 0.0379x + 0.0301	3.137	21.96 ± 6.79
	HE.Extract		8.665	60.65 ± 0.13
Lippia multiflora	Aq.Extract		3.862	27.04 ± 4.04
	HE.Extrait		6.343	44.40 ± 1.43
Phyllanthus amarus	Aq.Extrait		4.205	29.44 ± 1.17
	HE.Extrait		9.588	67.11 ± 0.39

3.3.2. Total Flavonoid Content

Flavonoid content was determined using Quercetin as the standard, expressed as milligram Quercetin equivalent per gram extract (mgeqQ/g).

On the other hand, the results indicate a higher quantity of flavonoids in the hydro-ethanolic extracts of all three plants than in their aqueous extracts. However, the hydro-ethanol extract of *Lippia multiflora* (38.88 ± 0.29) is richer in flavonoids than that of *Phyllanthus amarus* (31.79 ± 0.43) and *Catharanthus roseus* (26.96 ± 1.74).

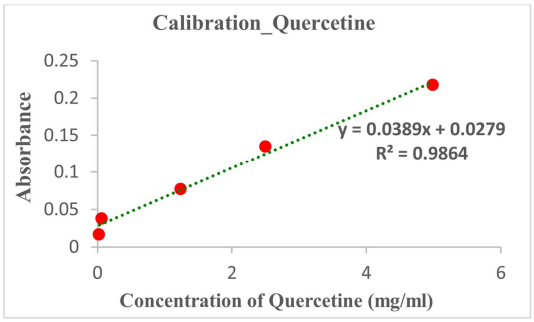

Figure 7. *Quercetine calibration curve.*

Table 5. *Total flavonoid content of aqueous and hydro-ethanol plant extracts.*

Plants	Extracts	Calibration Equation	Concentrations	Contents (mgeqQ/g)
Catharanthus roseus	Aq.Extract	y = 0.0389x + 0.0279	1.442	11.53 ± 0.29
	HE.Extract		3.37	26.96 ± 1.74
Lippia multiflora	Aq.Extract		1.005	8.04 ± 0.29
	HE.Extract		4.861	38.88 ± 0.29
Phyllanthus amarus	Aq.Extract		1.326	10.61 ± 0.43
	HE.Extract		3.974	31.79 ± 0.43

3.3.3. Total Tannin Content

Levels were reported in micrograms of Catechin equivalent per milligram of plant material extract.

Overall, the results indicate higher tannin content in the hydro-ethanolic extracts of all three plants than in their aqueous extracts. However, the hydro-ethanol extract of *Lippia multiflora* (3.62 ± 0.02) is richer in flavonoids than that of *Phyllanthus amarus* (2.67 ± 0.01) and *Catharanthus roseus* (2.16 ± 0.002).

Table 6. *Total tannin content of aqueous and hydro-ethanol plant extracts.*

Plants	Extracts	Calibration Equation	Concentrations	Contents (µgeCAT/g)
Catharanthus roseus	Aq.Extract	y = 0.0057x + 0.56	193.245	1.73 ± 0.01
	HE.Extract		240	2.16 ± 0.002
Lippia multiflora	Aq.Extract		176.491	1.58 ± 0.004
	HE.Extract		402.456	3.62 ± 0.02
Phyllanthus amarus	Aq.Extract		149.385	1.34 ± 1.08
	HE.Extract		296.666	2.67 ± 0.01

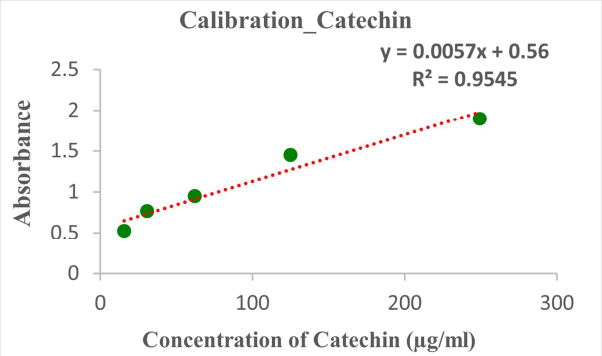

Figure 8. *Catechin calibration curve.*

The presence of phenols, flavonoids and tannins in the various aqueous and hydro-ethanolic extracts of the species analysed is an important indicator of hypoglycemic or antidiabetic activity for these plant species. Past studies have suggested that these secondary metabolites are endowed with hypoglycemic and antidiabetic properties [32]. A diet rich in polyphenols (phenolic acids, anthocyanin flavonols, etc...) would improve insulin sensitivity in obese or overweight individuals and even in non-diabetics [33]. Similarly, the anti-diabetic action of tannins is signalled by their action on diabetes itself at the cellular level, promoting insulin action (by reducing insulin resistance) and on diabetic complications through their antioxidant and anti-enzymatic power, neutralizing the effect of free radicals and limiting the inflammatory reaction in the various tissues.

3.4. Anti-radicalar Activity of Plant Extracts

3.4.1. Antioxidant Selection and Stability

Antioxidant selection and stability involves optimizing the molecular structure of each oxidant using B3LYP exchange-correlation functional [34, 35] of DFT (Density Functional Theory) and quantum basis set 6-31G (d, p) [36], in order to exalt the density of the electron sequence. All the calculations are carried out with Gaussian16W software package [37] at room temperature, combined with GaussView. 6.0.16 software for molecules and frontier molecular orbitals visualization [38]. The results show the energy minimum of the molecular structure in relation to its relative stability [39]. The electron density at the HOMO and LUMO occupied and unoccupied levels of the molecular orbitals exposes the state of relative stability in correlation with the energy minimum state of the molecules.

Comparing the relative stability energies of the antioxidants, it is noted that DPPH-H is the antioxidant with the highest energy (|E| = 890163.21 kcal/mol) and therefore presents a relative stability for a consequent trapping compared to the other antioxidants.

3.4.2. Analysis of Anti-radicalar Activity

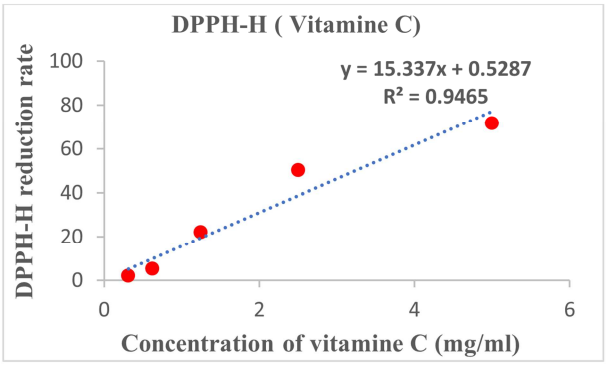

Figure 9. *Regression line for the rate of DPPH-H reduction by vitamin C as a function of concentration.*

The antioxidant capacity of the extract is measured in terms of radical scavenging capacity by tracking the reduction in absorbance of a hydro-ethanol solution of DPPH-H. After spectrophotometric measurement, the optical density values obtained made it possible to calculate radical inhibition percentages and to draw curves with a linear appearance, with the presence of a stationary phase signifying the almost total reduction of DPPH° to its non-radical form DPPH-H. From these curves we determined the IC50 value for the extracts [40]. The smaller the IC50 value, the better the antioxidant activity of the extracts [41]. Activity is considered as a decrease in the absorbance of the sample compared to the standard DPPH solution. The regression line expressing radical inhibition percentages as a function of ascorbic acid concentrations is shown in Figure 9 and antioxidant activity in Figure 10.

The results show that the percentage of DPPH-H reduction is proportional to substrate concentration for both vitamin C and extracts. More precisely, an increase in sample concentration provokes an increase in the percentage of free radical reduction, and consequently a strong antioxidant activity is exhibited.

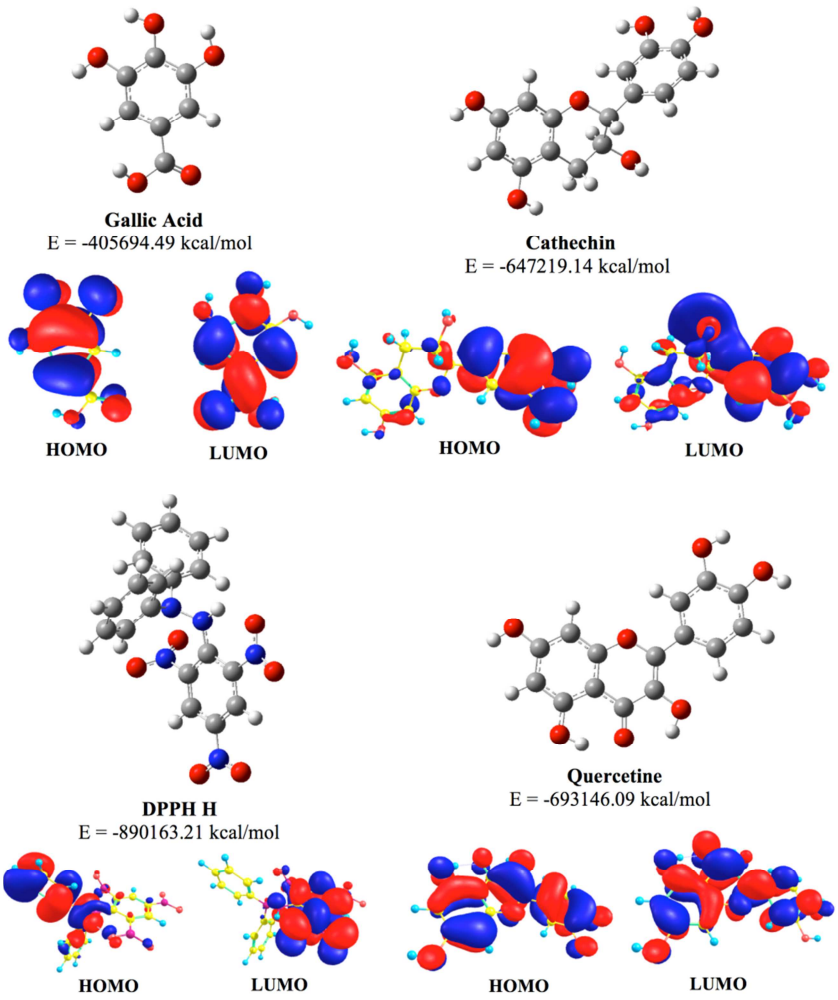

Figure 10. *Graphical diagrams of plant antioxidant molecules and their HOMO and LUMO molecular frontier orbitals, proving their stability.*

The antioxidant activity of the various extracts is expressed in IC50 values, which are shown in the following Figure 11:

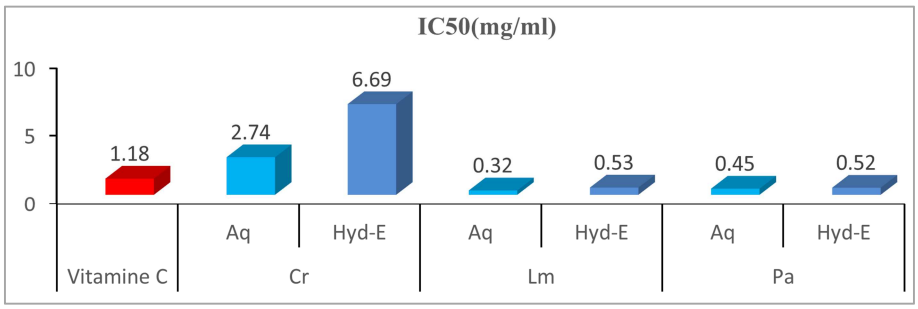

Figure 11. *IC50 (mg/ml): Vitamine C, Catharanthus roseus (Cr), Lippia multiflora (Lm) and Phyllanthus amarus (Pa).*

The results show that the leaves and flowers of *Lippia multiflora* (Lm) and *Phyllanthus amarus* (Pa) have very high anti-radicalar activity, superior to that of Vitamin C. Aqueous extracts of these plants are the most active, with IC50 values of 0.32 mg/ml and 0.45 mg/ml respectively, followed by their hydro-ethanol extracts with IC50 values of 0.53 mg/ml and 0.52 mg/ml respectively. With regard to the aqueous and hydro-ethanolic extracts of *Catharanthus roseus* (Cr), the results of our study demonstrate that they have a remarkable antioxidant potential, but more than that of the vitamin C standard.

On the whole, these plants, through their leaves and flowers, would therefore be useful as free radical scavengers, helping in the treatment of numerous diseases caused by reactive oxygen species. These include ageing, inflammation, cancer, atherosclerosis, hypertension and diabetes.

As the bibliography already contains data on the cytotoxicity of *Lippia multiflora* [42] and *Phyllanthus amarus* [43, 44], and given the high use of these plants by the population, it was of interest to us to assess the cytotoxicity of *Catharanthus roseus*, given the limited number of studies on it.

3.4.3. Cytotoxic Activity of Catharanthus roseus Extract

From the numbers of dead larvae recorded at the end of the test reading, we have plotted the variation curves of larval cell sensitivity as a function of different extract concentrations. Figures 12 and 13 respectively show the regression curve expressing the number of dead larvae as a function of the concentration of the aqueous extract and the hydro-ethanol extract of *Catharanthus roseus* leaves and flowers.

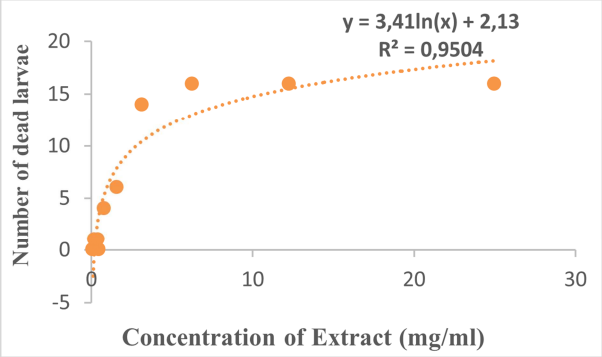

Figure 12. *Sensitivity curve of Artemia salina larvae to aqueous extract of Catharanthus roseus.*

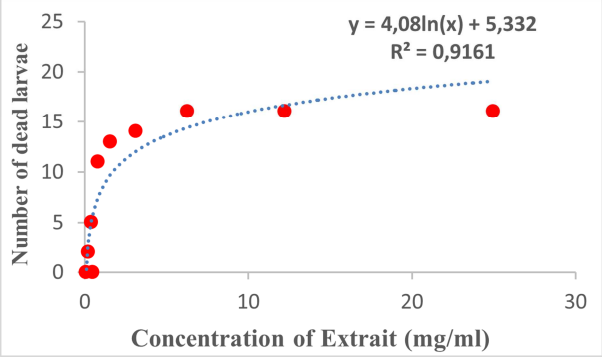

Figure 13. *Sensitivity curve of Artemia salina larvae to hydro-ethanol extract of Catharanthus roseus.*

For the graphs obtained, the R^2 correlation coefficient is greater than 0.8. There is therefore a good correlation between the concentrations applied and the responses obtained. Furthermore, larvae are sensitive to the extracts tested. The number of dead larvae increases with concentration, so larval sensitivity follows a dose-response relationship. From the above sensitivity curves, the value of the lethal half-concentration IC50 was calculated as 4.6792 mg/ml or 4679 µg/ml for the aqueous extract and 1.7774 mg/ml or 1777µg /ml for the hydro-ethanol extract. These values express the concentration necessary for the survival of 50% of the larval population introduced into the solutions tested.

Analysis of the IC50 values of the extracts tested against, allows us to state that the decoctate of *Catharantus roseus* roots is non-toxic in the range of concentrations analyzed, as the IC50 value obtained is at least 10 times higher than the set limit (0.1 mg/mL). The non-toxic nature of the extract revealed by the general toxicity test, justifies the results of the phytochemical screening, which showed the absence of cardiotonic heterosides, cyanogenic derivatives and quinonic derivatives, which are generally toxic compounds.

4. Conclusion

The present research proposed three Beninese medicinal plants with a major focus on diabetic diseases and given their many virtues that contribute to human system. The results of the present study has confirmed that the investigated plant species namely *Catharanthus roseus*, *Lippia multiflora* and *Phyllanthus amarus*, can be considered as wealthy resource of phyto-constituents, which can be isolated as well as examined for bio-efficacies and different pharmacological activities. Our study revealed also the presence of certain phenolic compounds (phenols, tannins and flavonoids) in the leaves and flowers of these medicinal plants. Our study also showed that extracting the plants resulted in a higher hydro-ethanol extraction yield than aqueous extraction. Thus, we were also able to confirm the richness of these plants in polyphenols, flavonoids and tannins. Although the results presented in our study confirm the potential of these plants for use in the pharmaceutical industry, in the fight against diabetes, we must nonetheless take an interest in them and explore them further in order to better exploit them, without forgetting to be aware of their domestic use.

In the next investigations, we will be able to tackle the Molecular Docking scores of the various active principles of these plants and carry out ADMET studies for biological properties to exalt and enhance the intrinsic properties and bioefficacy of these plants.

Declaration of Competing Interest

The authors declare no known competing financial interests.

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References

[1] Prabhakar P. K. & Doble M. (2011). Mechanism of action of natural products used in the treatment of diabetes mellitus. Chinese Journal of Integrative Medicine. 17 (8): 563–574. doi: 10.1007/s11655-011-0810-3.

[2] Padhi S., Nayak A. K. & Behera A. (2020). Type II diabetes mellitus: a review on recent drug based therapeutics. Biomedicine & Pharmacotherapy. 131: 110708. doi: 10.1016/j.biopha.2020.110708.

[3] Kesari A. N., Kesari S., Singh S. K., Gupta R. K. & Watal G. (2007) Studies on the glycemic and lipidemic effect of Murraya koenigii in experimental animals. Journal of Ethnopharmacology, 112 (2): 305–311. doi: 10.1016/j.jep.2007.03.023.

[4] Cho N. H., Shaw J. E., Karuranga S., Huang Y., da Rocha Fernandes J. D., Ohlrogge A. W. & Malanda B. (2018). IDF Diabetes Atlas: Global estimates of diabetes prevalence for 2017 and projections for 2045. Diabetes Research and Clinical Practice. 138: 271–281. doi: 10.1016/j.diabres.2018.02.023.

[5] Grover J. K., Yadav S. & Vats V. (2002). Medicinal plants of India with anti-diabetic potential. Journal of Ethnopharmacology. 81 (1): 81–100. doi: 10.1016/s0378-8741(02)00059-4.

[6] Luciano-Feijoó M., Cabañas J. G. & Brice J. H. (2021). Diabetic emergencies. Emergency Medical Services: Clinical Pratice and Systems Oversight. (1): 188–193. doi: 10.1002/9781119756279.ch20.

[7] Gambhire M. S., Gambhire V. M., Kulkarni A., Dolas R., Mulay A. & Bhide M. (2023). Design, Optimization and Characterization of Metformin Hydrochloride Loaded Biodegradable Microspheres using Box Behnken design for Local Delivery in Periodontitis. Int. J. Pharm. Sci. Drug. Res. 15 (3): 301-316. doi: 10.25004/IJPSDR.2023.150311.

[8] Gomes A., Coelho P., Soares R. & Costa R. (2021). Human umbilical cord mesenchymal stem cells in type 2 diabetes mellitus: the emerging therapeutic approach. Cell and Tissue Research. 385: 497-518. doi: 10.1007/s00441-021-03461-4.

[9] Fah L., Klotoé J. R., Dougnon V., Koudokpon H., Fanou V. B. A., Dandjesso C. & Loko F. (2013). An ethnobotanical study of plants used in the treatment of diabetes in pregnant women in Cotonou and Abomey-Calavi. Journal of Animal & Plant Sciences. 18 (1): 2647-2658. www.m.elewa.org/JAPS.

[10] Osadebe P. O., Odoh U. E. & Uzor P. (2014). Natural products as potential sources of antidiabetic drugs. British Journal of Pharmaceutical Research, 4 (17): 2075-2095. doi: 10.9734/BJPR/2014/8382.

[11] Michala A-S. & Pritsa A. (2022). Quercetin: A Molecule of Great Biochemical and Clinical Value and Its Beneficial Effect on Diabetes and Cancer. Diseases. 10 (3): 37. doi: 10.3390/diseases10030037.

[12] Andrade C., Gomes N. G. M., Duangsrisai S., Andrade P. B., Pereira D. M. & Valentão P. (2020). Medicinal plants utilized in Thai Traditional Medicine for diabetes treatment: Ethnobotanical surveys, scientific evidence and phytochemicals. J Ethnopharmacol. 5; 263: 113177. doi: 10.1016/j.jep.2020.113177.

[13] Thomas S. & Madhumitha G. (2022). Anti-diabetic Activity of Buchanania lanceolata Wight Extracts in Streptozocin persuades Diabetic Animal Model. Biointerface Research in Apllied Chemistry. 12 (5): 6741-6752. doi: 10.33263/BRIAC25.67416752.

[14] Chandrasekhar M., Syam Prasad G., Venkataramaiah C., Umapriya K., Raju C. N., Seshaiah K. & Rajendra W. (2020). In silico and in vitro antioxidant and anticancer activity profiles of urea and thiourea derivatives of 2,3-dihydro-1H-inden-1-amine. J Recept Signal Transduct Res, 40 (1): 34–41. doi: 10.1080/10799893.2019.1710848.

[15] Kotaiah Y., Harikrishna N., Nagaraju K. & Venkata Rao C. (2012). Synthesis and antioxidant activity of 1,3,4-oxadiazole tagged thieno [2,3-d]pyrimidine derivatives. European Journal of Medicinal Chemistry. 58: 340–345. doi: j.ejmech.2012.10.007.

[16] Kadali V. N., Pola S. R., Ramesh T. & Sandeep B. V. (2016). Anti-diabetic plants present in West Godavari district of Andhra Pradesh India- A short review. International Journal of Pharma Sciences and Research. 7 (2): 72-76. www.ijpsr.info/docs/IJPSR16-07-02-103.

[17] Biswajit S. & Bhabesh C. G. (2018). Evaluation of Hypoglycemic effect of Ethanol extract of Musa paradisiaca unripe fruit pulp on normal and alloxan induced diabetic mice. Research J. Pharm. and Tech. 11 (3): 1048-1052. doi: 10.5958/0974-360X.2018.00196.8.

[18] Nwonu C., Ilesanmi O., Agbedahunsi J. & Nwonu P. (2019). Natural products as veritable source of noveldrugs and medicines: A review. International Journal of Herbal Medicine. 7 (1): 50-54. www.florajournal.com/archives/2019/vol7issue1/PartA/7-2-44-652.

[19] Biswajit S. (2021). Phytochemical analysis of few selected medicinal plants used for the treatment of diabetes in Assam, India. Research Journal of Chemical Sciences. 11 (1): 34-41. www.isca.me/rjcs/Archives/v11/i1/6.ISCA-RJCS-2020-058.

[20] Cheng H. T., Xu X., Lim P. S. & Hung K. Y. (2021). Worldwide epidemiology of diabetes-related end-stage renal disease, 2000–2015. Diabetes Care. 44 (1): 89–97. doi: 10.2337/dc20-1913.

[21] Kong L. Y. & Tan R. X. (2015). Artemisinin, a miracle of traditional Chinese medicine. Natural Product Reports. 32 (12): 1617–1621. doi: 10.1039/c5np00133a.

[22] Houngbeme A. G., Ganfon H. M. Y., Medegan S., Yehouenou B., Bambola B., Gandonou C. & Gbaguidi F. A. (2015). Antimicrobial activity of compounds from Acanthospermum hispidum DC and Caesalpinia bonduc (L.) ROXB: Beninese plants used by healers against HIV-associated microbial infections. Journal of Applied Pharmaceutical Science. 5 (08): 073-081. doi: 10.7324/JAPS.2015.50812.

[23] Harborne J. B. (1998). Textbook of Phytochemical Methods. A Guide to Modern Techniques of Plant Analysis. 5th Edition, Chapman and Hall Ltd, London, 21-72.

[24] Fatoumata K. M., Moussa S., Housseini D., Ilo D., Abdoul F. D., Modibo K., Lamine D., Oumar D., Issa G., Mahamadou T., Ousmane F., Seydou D. & Yaya I. C. (2022). Knowledge and factors influencing schistosomiasis control interventions in the hyperendemic health district of Kalabancoro in Mali, 2020. Pan Afr Med J. 43 (48): 30512. doi: 10.11604/pamj.2022.43.48.30512.

[25] Kim E. J., Ahn B. K. & Kang C. W. (2003). Evaluation of the nutritive value of local defatted rice bran and effects of its dietary supplementation on the performance of broiler chicks. J. Anim. Sci. Technol. 45 (5): 759-766. doi: 10.5187/JAST.2003.45.5.759.

[26] Kaur C. & Kapoor H. (2002). Anti-Oxidant Activity and Total Phenolic Content of Some Asian Vegetables. International Journal of Food Science and Technology. 37: 153-161. doi: 10.1046/j.1365-2621.2002.00552.x.

[27] Durand D-N., Hubert A-S., Nafan D., Haziz S., Adolphe A., Mariam I., Donald A., Joachim D. G., Simeon O. K., Mamoudou H. D. & Lamine, B-M. (2015). Phytochemical Analysis and Biological Activities of Cola nitida Bark. Biochem. Res. Int. 493879. doi: 10.1155/2015/493879.

[28] Heimler D., Vignolini P., Dini M. G., Vincieri F. F. & Romani A. (2006). Antiradical activity and polyphenol composition of local Brassicaceae edible varieties. Food Chemistry. 99 (3): 464–469. doi: 10.1016/j.foodchem.2005.07.

[29] Lamien-Meda A., Lamien C. E., Compaoré M. M. Y., Meda R. N. T., Kiendrebeogo M., Zeba B., Millogo J. F. & Nacoulma O. G. (2008). Polyphenol Content and Antioxidant Activity of Fourteen Wild Edible Fruits from Burkina Faso. Molecules. 13 (3): 581–594. doi: 10.3390/molecules13030581.

[30] Gandonou C. D., Tokoudagba J-M., Houngbeme A. G., Chodaton M. D. & Ahissou H. (2018). Antiradical activity and determination of phenolic compounds of extracts of Lippia multiflora (Verbenaceae): a plant traditionally used against arterial hypertension in Benin. International Journal of Current Research. 10 (10): 74039-74043. doi: 10.24941/ijcr.32498.10.2018.

[31] Oyedeji S. I., Bassi P. U., Oyedeji S. A., Ojurongbe O. & Awobode H. O. (2020). Genetic diversity and complexity of Plasmodium falciparum infections in the microenvironment among siblings of the same household in North-Central Nigeria. Malar J. 19: 338. doi: 10.1186/s12936-020-03415-1.

[32] Jean de Dieu M. M., Félicien M. K. & Justin K. N. (2014). Contribution à l'étude phytochimique de quelquesplantes médicinales antidiabétiques de la ville de Bukavu et ses environs (Sud-Kivu, R. D. Congo). J. Appl. Biosci. 75: 6211-6220. doi: 10.4314/jab.v75i1.7.

[33] Paquette D., Bigras M. & Parent S. (2001). La validation du QSA et la prévalence des styles d'attachement adulte dans un échantillon francophone de Montréal [Validation of the ASQ and the prevalence of the styles of adult attachment in a French-speaking sample of Montreal]. Canadian Journal of Behavioural Science/Revue canadienne des sciences du comportement. 33 (2): 88–96. doi: 10.1037/h0087131.

[34] Becke A. D. (1993). Density-functional thermochemistry. III. The role of exact exchange. J. Chem. Phys. 98 (7): 5648–5652. doi: 10.1063/1.464913.

[35] Lee C., Yang W. & Parr R. G. (1988). Development of the Colle-Salvetti correlation-energy formula into a functional of the electron density. Phys. Rev. B. 37 (2): 785–789. doi: 10.1103/PhysRevB.37.785.

[36] Frisch M. J., Pople J. A. & Binkley J. S. (1984). Self-Consistent molecular orbital methods 25. Supplementary functions for Gaussian basis sets. J. Chem. Phys. 80 (7): 3265–3269. doi: 10.1063/1.447079.

[37] Frisch M. J., Trucks G. W., Schlegel H. B., Scuseria G. E., Robb M. A., Cheeseman J. R., Scalmani G., Barone V., Petersson G. A., Nakatsuji H., et al. (2016). Gaussian 16, Revision B.01, Gaussian, Inc., Wallingford CT.

[38] Dennington R., Keith T. & Millam J. (2019). GaussView, Version 6.0.16, Semichem Inc., Shawnee Mission, KS.

[39] Farhadi F., Khameneh B., Iranshahi M. & Iranshahy M. (2019). Antibacterial activity of flavonoids and their structure–activity relationship: An update review. Phytotherapy Research. 33: 13–40. doi: 10.1002/ptr.6208.

[40] Gnintoungbe G., Medehouenou T., Adounkpe F., Akpovi C. & Loko F. (2023). Phytochemical Screening, Antioxidant Activity and Safety of Petroselinum crispum (Mill.) AW Hill Apiaceae Leaves Grown in Benin. Open Journal of Applied Sciences. 13: 36-50. doi: 10.4236/ojapps.2023.131004.

[41] Bortolomeazzi R., Sebastianutto N., Toniolo R. & Pizzariello A. (2007). "Comparative evaluation of the antioxidant capacity of smoke flavouring phenols by crocin bleaching inhibition, DPPH radical scavenging and oxidation potential." Food Chemistry. 100 (4): 1481–1489. doi: 10.1016/j.foodchem.2005.11.039.

[42] Barrachina L., Cequier A., Romero A., Vitoria A., Zaragoza P., Vazquez F. J. & Rodellar C. (2020). Allo-antibody production after intraarticular administration of mesenchymal stem cells (MSCs) in an equine osteoarthritis model: effect of repeated administration, MSC inflammatory stimulation, and equine leukocyte antigen (ELA) compatibility. Stem Cell Res Ther. 11 (1): 52. doi: 10.1186/s13287-020-1571-8.

[43] Kamgaing M. T. W., Mvondo M. A., Kamani S. L. P., Essono S. M. & Ngnokam S. L. W. (2020). The Aqueous Extract of Dacryodes edulis (Burseraceae) Leaves Inhibits Cell Proliferation Induced by Estradiol on the Uterus and Vagina of Ovariectomized Female Wistar Rats. Adv Pharmacol Pharm Sci. (20): 8869281. doi: 10.1155/2020/8869281.

[44] Patel A., McKnight J. N., Genzor P. & Bowman G. D. (2011). Identification of residues in chromodomain helicase DNA-binding protein 1 (Chd1) required for coupling ATP hydrolysis to nucleosome sliding. J Biol Chem. 286 (51): 43984-43993. doi: 10.1074/jbc.M111.282970.