

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

Simplice Koudjina<sup>1,3,\*</sup>, Alban Gouton Hounbeme<sup>2</sup>, Israël Dossou Paulin Agbogba<sup>1</sup>,  
Guy Yacolé Sylvain Atohoun<sup>3</sup>, Joachim Djimon Gbenou<sup>4</sup>

<sup>1</sup>Laboratory of Medical Biotechnology and Pharmaceutical Research (LaRBiMeP), National High School of Applied Biosciences and Biotechnologies (ENSBB), National University of Sciences, Technologies, Engineering and Mathematics (UNSTIM), Abomey, Benin

<sup>2</sup>Laboratory of Pharmaceutical Organic Chemistry, UFR Pharmacy, Faculty of Health Sciences, University of Abomey-Calavi (UAC), Abomey-Calavi, Benin

<sup>3</sup>Laboratory of Chemical Physics-Materials and Molecular Modeling (LCP3M), University of Abomey-Calavi (UAC), Abomey-Calavi, Benin

<sup>4</sup>Laboratory Pharmacognosies and Essential Oils (LAPHE), University of Abomey-Calavi (UAC), Abomey-Calavi, Benin

## Email address:

[simplice.koudjina@unstim.bj](mailto:simplice.koudjina@unstim.bj) (Simplice Koudjina), [albanusphd@yahoo.fr](mailto:albanusphd@yahoo.fr) (Alban Gouton Hounbeme),  
[agbgbaisrael@gmail.com](mailto:agbgbaisrael@gmail.com) (Israël Dossou Paulin Agbogba), [agys2000@yahoo.fr](mailto:agys2000@yahoo.fr) (Guy Yacolé Sylvain Atohoun),  
[gjdjim@yahoo.fr](mailto:gjdjim@yahoo.fr) (Joachim Djimon Gbenou)

\*Corresponding author

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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  $60.65 \pm 0.13$ ;  $44.40 \pm 1.43$  and  $67.11 \pm 0.39$  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

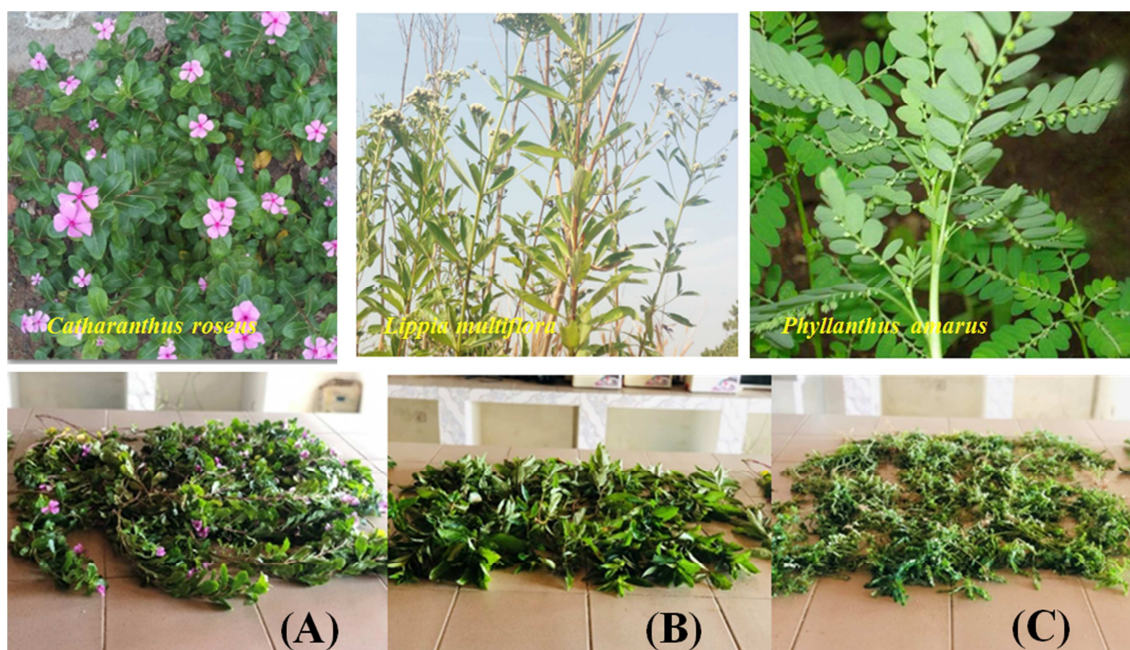
In the traditional treatment of diseases, medicinal plants, through their leaves and flowers play an essential role by displaying different biological activities and bioefficacy. Metabolic and pathologic disease related to Diabetes, one of the major degenerative chronic diseases in the world characterized 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, uncomfortable 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 phytochemical 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-radical 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.

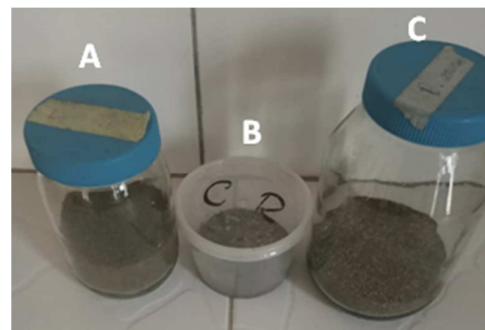


Figure 2. Powders of *L. multiflora* (A), *C. roseus* (B), *P. amarus* (C).



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<br>- Mayer (potassium iodomercurate) → yellowish precipitate                                                                                            |
| Tannins                  | - $\text{FeCl}_3$ → 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 ( $\text{EtOH } 50^\circ/\text{HCl}_{cc} 2: 1$ ) → cherry red coloring                                                                                                                                   |
| Quinonic derivatives     | - Bornträger (reaction between quinonic rings in a medium $\text{NH}_4\text{OH}$ ) → purplish-red color                                                                                                               |
| Saponosides              | - Determination of foam index (positive if $\text{IM} > 100$ )                                                                                                                                                        |
| Steroids and Terpenes    | - Liebermann-Burchard (Acetic anhydride- $\text{H}_2\text{SO}_{4cc} 50: 1$ ) → violet coloring<br>- Kedde (dinitrobenzoic acid 1% in $\text{EtOH} + \text{NaOH } 1\text{N } 1: 1$ ) → 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:

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

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<sub>2</sub>CO<sub>3</sub> at 75 mg/ml is added. The mixture is vortexed 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:

$$T \left( \frac{\text{mg eq GA}}{\text{g}} \right) = \frac{C \cdot V_r}{V_p \cdot C_p} \quad (2)$$

Where, T = Compounds content, C = Concentration obtained from calibration curve, V<sub>r</sub> = Reaction volume, V<sub>p</sub> = Volume of extract, C<sub>p</sub> = Concentration of sample extract.

### 2.2.4. Determination of Flavonoids Content

Take 500 µL of AlCl<sub>3</sub> 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<sub>3</sub> 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 Quercetin (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 Quercetin (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 (λ<sub>max</sub> DPPH•) [28, 29]. The initially violet DPPH• is transformed into the pale yellow DPPH-H.

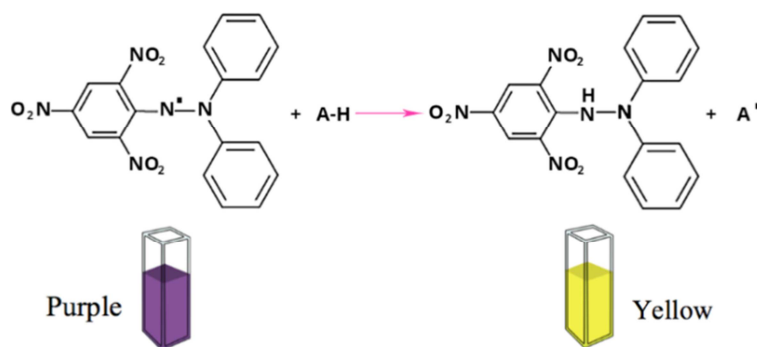


Figure 5. Antioxidant activity reaction pathway by using DPPH• test.

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• methanolic 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:

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

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 IC<sub>50</sub> 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.

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

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

MI: Initial mass,  $\Delta m$ : 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           |                      |                  |
|--------------------------|------------------------|--------------------|------------------|----------------------|------------------|
|                          |                        |                    | <i>C. roseus</i> | <i>L. multiflora</i> | <i>P. amarus</i> |
| Phenolic compounds       | Tannins                | Catechic tannins   | +                | -                    | +                |
|                          |                        | Gallic tannins     | +                | +                    | +                |
|                          | Flavonoïdes            | Flavones           | +                | +                    | +                |
|                          |                        | Leuco-anthocyanins | +                | -                    | +                |
|                          | Anthocyanins           |                    | +                | +                    | -                |
|                          |                        | Free               | -                | -                    | -                |
|                          | Anthracene derivatives | O-heteroside       | -                | -                    | +                |
|                          |                        | Combination        | -                | -                    | -                |
|                          |                        | 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,

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].

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 ( $29.44 \pm 1.17$ ) and hydro-ethanolic ( $67.11 \pm 0.39$ ) extracts of *Phyllanthus amarus* have a higher polyphenol content than those of *Lippia multiflora* ( $27.04 \pm 4.04$  and  $44.40 \pm 1.43$ ) and *Catharanthus roseus* ( $21.96 \pm 6.79$  and  $60.65 \pm 0.13$ ).

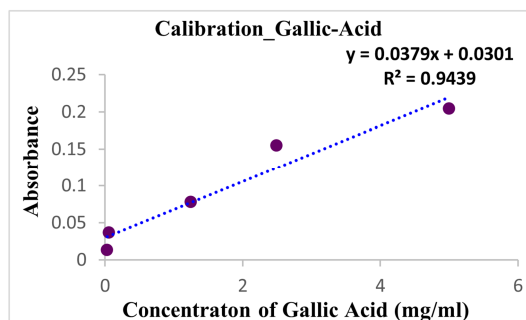


Figure 6. Gallic acid calibration curve.

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

| Plants                     | Extracts   | Calibration Equation   | Concentrations | Contents (mgeqAG/g) |
|----------------------------|------------|------------------------|----------------|---------------------|
| <i>Catharanthus roseus</i> | Aq.Extract | $y = 0.0379x + 0.0301$ | 3.137          | $21.96 \pm 6.79$    |
|                            | HE.Extract |                        | 8.665          | $60.65 \pm 0.13$    |
| <i>Lippia multiflora</i>   | Aq.Extract |                        | 3.862          | $27.04 \pm 4.04$    |
|                            | HE.Extrait |                        | 6.343          | $44.40 \pm 1.43$    |
| <i>Phyllanthus amarus</i>  | Aq.Extrait |                        | 4.205          | $29.44 \pm 1.17$    |
|                            | HE.Extrait |                        | 9.588          | $67.11 \pm 0.39$    |

#### 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* ( $38.88 \pm 0.29$ ) is richer in flavonoids than that of *Phyllanthus amarus* ( $31.79 \pm 0.43$ ) and *Catharanthus roseus* ( $26.96 \pm 1.74$ ).

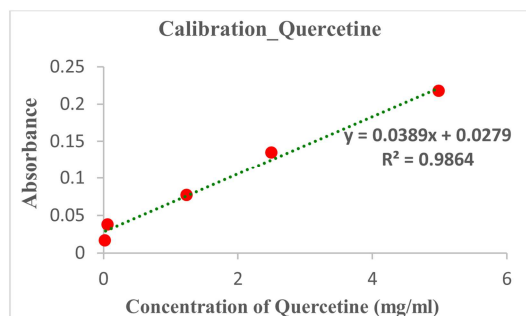


Figure 7. Quercetine calibration curve.

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

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

#### 3.3.3. Total Tannin Content

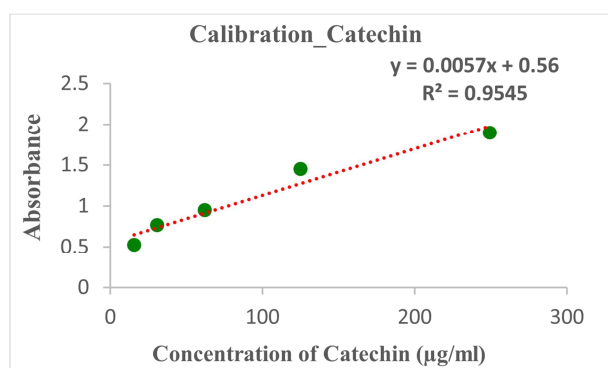
Levels were reported in micrograms of Catechin equivalent per milligram of plant material extract.

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* ( $3.62 \pm 0.02$ ) is richer in flavonoids than that of *Phyllanthus amarus* ( $2.67 \pm 0.01$ ) and *Catharanthus roseus* ( $2.16 \pm 0.002$ ).

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

| Plants                     | Extracts   | Calibration Equation | Concentrations | Contents ( $\mu\text{gCAT/g}$ ) |
|----------------------------|------------|----------------------|----------------|---------------------------------|
| <i>Catharanthus roseus</i> | Aq.Extract | $y = 0.0057x + 0.56$ | 193.245        | $1.73 \pm 0.01$                 |
|                            | HE.Extract |                      | 240            | $2.16 \pm 0.002$                |
| <i>Lippia multiflora</i>   | Aq.Extract |                      | 176.491        | $1.58 \pm 0.004$                |
|                            | HE.Extract |                      | 402.456        | $3.62 \pm 0.02$                 |
| <i>Phyllanthus amarus</i>  | Aq.Extract |                      | 149.385        | $1.34 \pm 1.08$                 |
|                            | HE.Extract |                      | 296.666        | $2.67 \pm 0.01$                 |

**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.

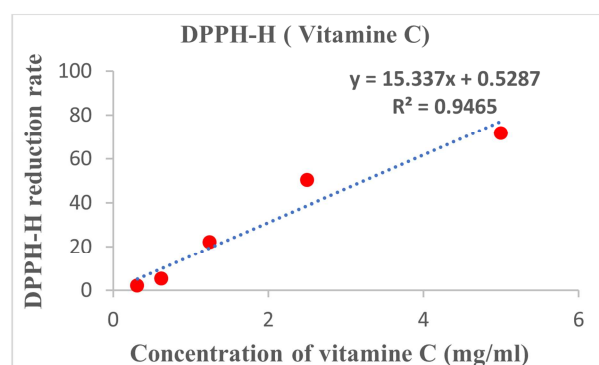
### 3.4. Anti-radical Activity of Plant Extracts

#### 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.

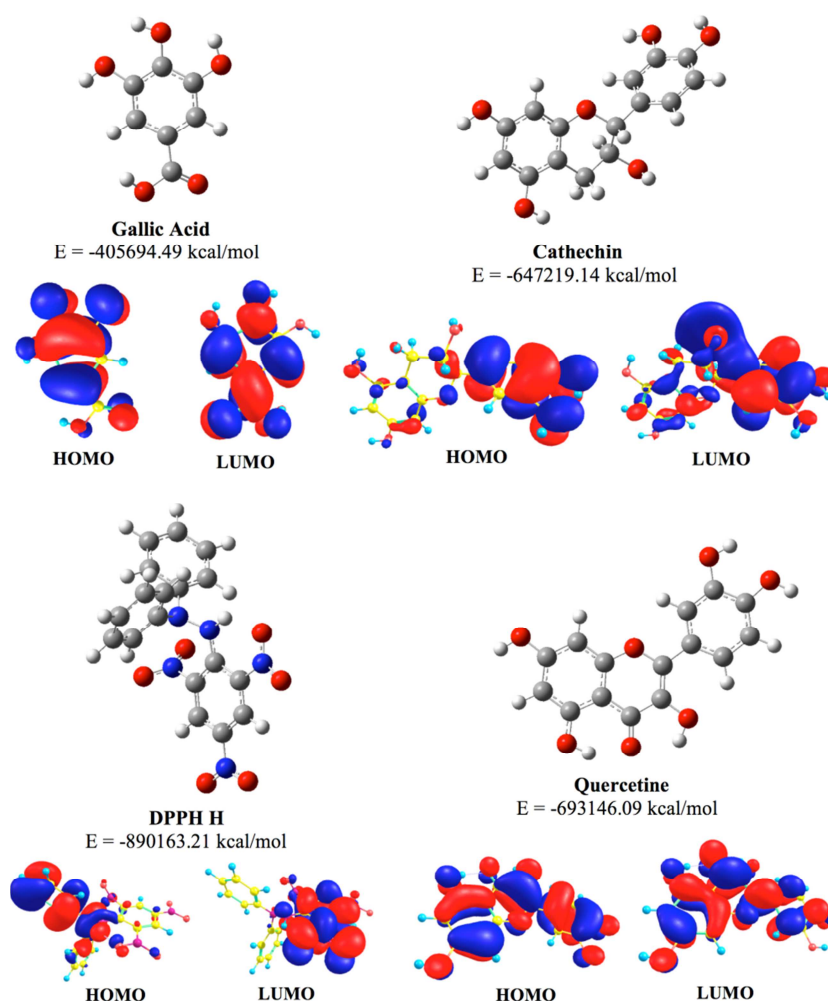
Comparing the relative stability energies of the antioxidants, it is noted that DPPH-H is the antioxidant with the highest energy ( $|E| = 890163.21$  kcal/mol) and therefore presents a relative stability for a consequent trapping compared to the other antioxidants.

#### 3.4.2. Analysis of Anti-radical Activity

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

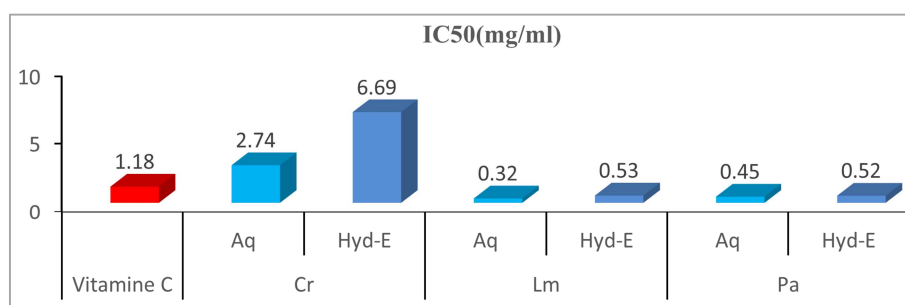
The antioxidant capacity of the extract is measured in terms of radical scavenging capacity by tracking the reduction in absorbance of a hydro-ethanol solution of DPPH-H. After spectrophotometric measurement, the optical density values obtained made it possible to calculate radical inhibition percentages and to draw curves with a linear appearance, with the presence of a stationary phase signifying the almost total reduction of  $\text{DPPH}^\bullet$  to its non-radical form DPPH-H. From these curves we determined the IC<sub>50</sub> value for the extracts [40]. The smaller the IC<sub>50</sub> 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 IC<sub>50</sub> values, which are shown in the following Figure 11:



**Figure 11.** IC<sub>50</sub> (mg/ml): Vitamine C, *Catharanthus roseus* (Cr), *Lippia multiflora* (Lm) and *Phyllanthus amarus* (Pa).

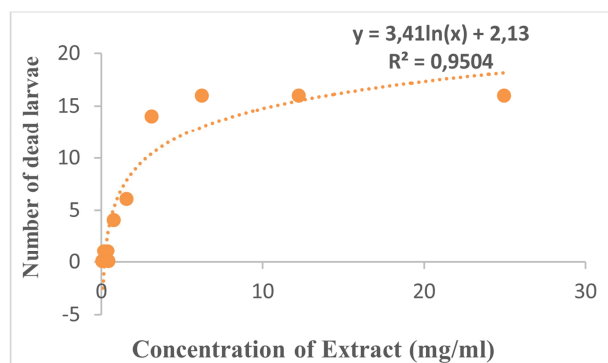
The results show that the leaves and flowers of *Lippia multiflora* (Lm) and *Phyllanthus amarus* (Pa) have very high anti-radical activity, superior to that of Vitamin C. Aqueous extracts of these plants are the most active, with IC<sub>50</sub> values of 0.32 mg/ml and 0.45 mg/ml respectively, followed by their hydro-ethanol extracts with IC<sub>50</sub> 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.

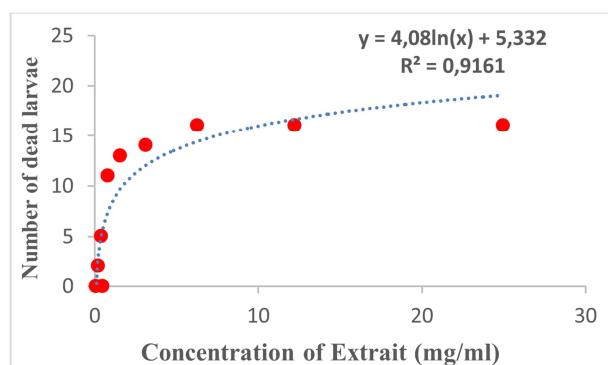
As the bibliography already contains data on the cytotoxicity of *Lippia multiflora* [42] and *Phyllanthus amarus* [43, 44], and given the high use of these plants by the population, it was of interest to us to assess the cytotoxicity of *Catharanthus roseus*, given the limited number of studies on it.

### 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  $IC_{50}$  was calculated as 4.6792 mg/ml or 4679  $\mu$ g/ml for the aqueous extract and 1.7774 mg/ml or 1777  $\mu$ g /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  $IC_{50}$  values of the extracts tested against, allows us to state that the decoctate of *Catharanthus roseus* roots is non-toxic in the range of concentrations analyzed, as the  $IC_{50}$  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 cardiotoxic 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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