Phytochemical screening, antioxidant activity and cytotoxicity of four medicinal plants for antidiabetic purposes used in the Ivorian pharmacopoeia

ABSTRACT

Objectives:The aim of this study is to defineat first timethe phytochemical profile of four plants used in the District of Yamoussoukro (Côte d'Ivoire) for the treatment of diabetes and to evaluate their antioxidant capacity. In a second step, this study was based on evaluating the toxicity of their extract and verifying the effectiveness of their antidiabetic activity. These plants are *Alchornea cordifolia, Ocimumgratissimum, Tetrapleura tetraptera* and *Vernonia colorata*.

Methodology:Secondary metabolites were identified by phytochemical screening. The polyphenol and tannin contents were determined by the colorimetric method of Folin-ciocalteu. The evaluation of the antioxidant activity of the different extracts was carried out *in vitro*by the DPPH (1,1-diphenyl-2-picryl-hydrazyl) test. In addition, acute toxicity was assessed by administering oral extracts to mice at single escalating doses. Hypoglycemic and antihyperglycemic activities were assessed by controlling the blood glucose levels of mice after administration of the extracts.

Results:Phytochemical screening revealed the presence of polyphenols and particularly flavonoids as well as alkaloids, saponosids, and terpens and sterols in all extracts. The highest total flavonoid content was obtained with *V. colorata* extract (58.10 mg/g QE DE). *A. cordifolia* extract has the highest content of total polyphenols (57.56 ± 2.34 mg/g GAEDE) and total tannins (0.84 ± 0.02 mg/g TAEDE). Also, the most important antioxidant capacity (0.4190 mg/mL) was observed with the extract of *A. cordifolia* as well as a good hypoglycemic activity at the dose of 300 mg/kg BW. In addition, all the extracts studied have a lethal dose greater than 5000 mg/kg BW.

Conclusion:These results show that the plant extracts studied contain several secondary metabolites responsible for their good antioxidant capacities. In addition, these plants studied have good antidiabetic activities and are not toxic by oral route. This could justify their use in traditional medicine in the fight against diabetes.

***Keywords**: medicinal plants; phytochemistry; toxicity; antioxidant; antidiabeticproperties*

1.INTRODUCTION

Diabetes is a disease of endocrine disorders and metabolites characterized by chronic hyperglycemia. It is caused either by a disruption in insulin secretion or function or both**[1]**. It increases the risk of complications from cardiovascular disease**[1]**, **[2]** and poses a real public health problem. Indeed, diabetes is the third most common chronic disease after cancer and cardiovascular disease. According to the IDF, in 2017, there were 451 million people with diabetes worldwide. This figure rose to 536 million in 2021 and is projected to reach 783 million by 2045**[3]**, **[4]**.

Despite the presence of antidiabetic drugs on the pharmaceutical market, herbal diabetes treatment is practiced by more than 80% of the rural population. For centuries, plants have been considered a fundamental source of medicines for health care. In developing countries, in general, herbal medicines are used to treat diabetes to palliate the high costs and accessibility of conventional medicines for the low-income population**[5]**. Nowadays, for the treatment of several conditions including diabetes, the use of medicinal plants is recommended by Lee et al.,**[6]**. Indeed, they contain various secondary metabolites including polyphenols, terpenoids, saponins, alkaloids and glycosides with antidiabetic properties**[7]** and without significant side effects**[8]**.

In response to the high level of coverage of modern medicines and the side effects of their prolonged use, WHO, in its resolution AFR/RC50/R3 of 31 August 2000, encourages African countries to develop regional strategies on traditional medicine in order to undertake research on medicinal plants and promote their optimal uses in health-care delivery systems. Among these plants are *Alchornea cordifolia, Ocimumgratissimum, Tetrapleura tetraptera* and *Vernonia colorata*which are used in the district of Yamoussoukro for the treatment of diabetes.

The objective of this study is to define the phytochemical profile of these frequently used plant drugs, to evaluate their antioxidant capacity, toxicity as well as their hypoglycemic and antihyperglycemic activity for better management of diabetic patients.

2. MATERIAL AND METHODS

2.1Plant material

The plant material consists of the leaves of *Alchornea cordifolia* and *Vernonia colorata*, the whole plant of *Ocimumgratissimum* collected in Djahakro and Kami, villages located in the locality of Yamoussoukro (Center of Côte d'Ivoire) and the fruits of *Tetrapleura tetraptera* harvested in Sikensi in the Agnéby-Tiassa region(South of Côte d'Ivoire). These plant species were identified by the botanist N'GUESSAN Amani in accordance with the herbaria available at the Higher School of Agronomy of the National Polytechnic InstituteHouphouëtBoignyof Yamoussoukro.

After harvest, the plant material was sent to the laboratory to be dried at room temperature for 14 days. Once dried, the various plant organs were crushed.

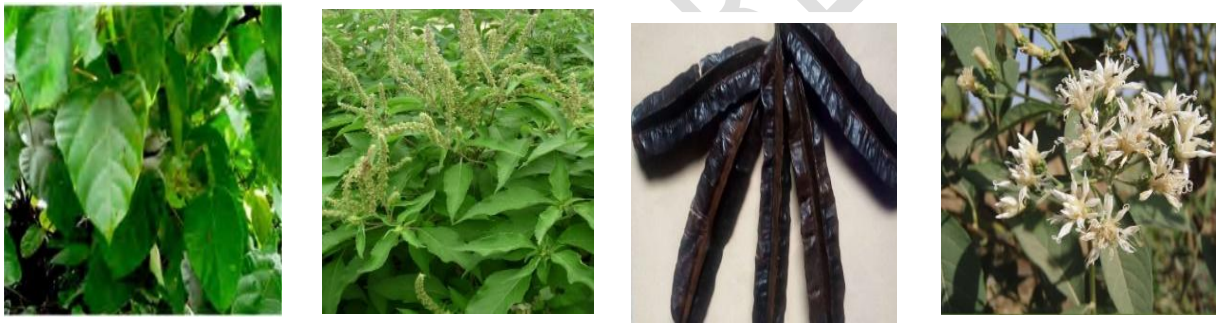

Fig 1 :A (*Alchornea Cordifolia*), B (*Ocimumgratissimum*), C (*Tetrapleura tetraptera*) and D (*Vernonia colorata*)

2.1.1 Preparation of plants extracts

The extracts were obtained from the powders of dried samples according to the method described by Bidié et al.,**[9]**. 100g of each powder was mixed with 1L of distilled water. The whole was heated to reflux for 15 min. After that, the mixture was cooled and then filtered with Whatman paper. The filtrate obtained was dried at 55 ° C to obtain the dry extract (DE).

2.2 Animal material

Mice of the species Mus musculus, of Swiss strain, were used for the realization of the various in vivo experiments. They were caged in groups of 5 with a light/dark cycle of 12/12 hours and an ambient temperature of 28 ± 2 °C. These animals were fed with pellets from the Ivorian Compound Feed Manufacturing Company (FACI) and watered with tap water without interruption.
Female mice were used for the oral route acute toxicity test. They were nulliparous and not gravid. They were 8 weeks old and their body weight (BW) was between 19 and 21 g.
Male mice were used for testing hypoglycemic and antihyperglycemic activities. Their age ranged from 9 to 10 weeks and their BW ranged from 25 to 28 g.

2.3 Identification of phytochemical groups of extracts

Differentfamilies of secondary metabolites such as polyphenols, flavonoids, leuko-anthocyanins, tannins, saponosids, alkaloids, quinones, sterols/terpens, have been demonstrated in the extracts according to the method described by Bagreet al.**[10]**.

2.4 Determination of total polyphenols content

The determination of total polyphenols was carried out using the colorimetric method using the Folin-Ciocalteu reagent as described by Wood et al.,**[11]**. In a test tube containing 30 µL of extract was added 2.5 mL of Folin-Ciocalteu reagent diluted to 1/10th. The mixture is left in the dark for 2 minutes at room temperature (30±2°C). Then 2 mL of a 7.5% sodium carbonate solution was added. This mixture was placed in a water bath maintained at 50 ° C for 15 minutes, then cooled quickly. The absorbance was measured with a UV/visible spectrophotometer at a wavelength of 760 nm against a blank prepared under the same conditions. Gallic acid was used as a standard. The total polyphenol content was expressed in milligrams per literof gallic acid equivalent of extract (mg/L GAE).

2.5 Determination of total flavonoids content

The determination of total flavonoids was performed according to Marinova et al.**[12]**. In a 25 mL vial, 0.75 mL of 5% (w/v) sodium nitrite ($NaNO_2$) was added to 2.5 mL of extract. The mixture was added 0.75 mL of aluminum chloride ($AlCl_3$) at 10% (w/v), then incubated for 6 minutes in the dark. After incubation, 5 mL of sodium hydroxide (NaOH 1N) were added and the volume was completed to 25 mL. After vigorous agitation of the mixture, absorbance was measured with a UV-visible spectrophotometer at wavelength ʎ= 510 nm. The flavonoid content was expressed in milligramsper literquercetin equivalentof extract (mg/L QE). A calibration line was performed with quercetin at different concentrations.

2.6 Determination of total tannins content

The determination of total tannins was carried out by the Folin Ciocalteu reagent colorimetric method as described by Chandran et Indira**[13]**. 100 µL of extract were added to a test tube containing 7.5 mL of distilled water and 0.5 mL of Folin Ciocalteu's reagent. Then 1 mL of 35% Na_2CO_3(w/v) is added. The volume is completed to 10 mL by adding 900 µL of distilled water. The reaction mixture is incubated for 30 min at laboratory temperature (25–30°C). The absorbances are read with the UV/visible spectrophotometer at 700nm against the distilled water used as blank. The contents are expressed in micrograms per literof tannic acid equivalent of extract (mg/L TAE).

2.7 Evaluation of antioxidant activity (AAO) of extracts by DPPH

The measurement of the anti-radical activity of the extracts was carried out by the 2,2'-diphenyl-1-picrylhydrazyl (DPPH) test according to the method described by Parejo et al. **[14]**with some modifications. A range of concentrations of the extract or standard is prepared in an ethanol/water mixture (70/30) (v/v). A volume of 100 µL of this solution is mixed with 3.9 mL of DPPH (70 µM) prepared in ethanol in a test tube. After homogenization, the reaction mixture is incubated at room temperature (25 °C) away from light. Then 30 minutes of incubation, the absorbance is read at 517 nm against a blank containing only methanol. The percentage of inhibition of the DPPH radical is calculated according to the following equation:

DPPH inhibition (%) = $(A_0 - Ae)/A_0) \times 100$.

With A_0: Absorbance of the sample
Ae: Absorbance of the control

The CI50 values were estimated to be from the inhibition percentage curve in relation to the concentration.

2.8 Evaluation of the acute toxicity of extracts

Acute toxicity was achieved following a sequential process in which 3 mice were used at each step according to OECD Test Guideline 423 **[15]**. A determined dose of the extract is administered by oral route to a group of animals. The absence or manifestation of extract-related mortality in a dose group

at a given stage determines the next step. This method determines the dose range at which the extract should be considered lethal. Following the absence of mortality and clinical signs at doses of 1000 and 2000 mg/kg BW, 15 female mice divided into 5 batches of 3 were used for the dose of 5000 mg/kg BW. The first batch received only distilled water (control batch). Each of the other four batches received an oral extract corresponding to the dose of 5000 mg/kg BW. The animals were then observed for 14 days.

2.9 Hypoglycemic activity in normoglycemic animals

The animals had previously fasted for 14 hours without water deprivation. Basal blood glucose was then measured prior to administration of the corresponding extracts to each batch. Blood glucose control was performed at 30, 60 and 120 minutes. The percentages of change in blood glucose levels were calculated at the different blood glucose measurement times.

2.10 Anti-hyperglycemic activities in animals subjected to glucose tolerance testing

After fasting for 14 hours without water restriction, basal blood glucose was then measured prior to administration of the corresponding extracts to each batch. The animals received at almost the same time (about 1 minute interval) the anhydrous glucose (4mg/kg of glucose BW) and then the different test substances. Blood glucose was monitored every 30 minutes for 180 minutes. The percentages of change in blood glucose levels were calculated at the different blood glucose measurement times.

2.11 Statistical analysis

Statistical analysis was performed by performing a one-factor analysis of variance (ANOVA) for all data (mean of each measured parameter). The different values obtained were expressed as an average followed by the standard mean error (M±ESM). Comparisons of means were performed by the Newman-Keuls test at the 5% significance level using GraphPad Prism 7 software.

3. RESULTS

3.1 Phytochemical composition of plant extracts studied

The phytochemicals of the plant extracts studied are summarized in Table I. Phenolic compounds, saponosids and sterols/terpens are present in all the extracts studied. In addition, all extracts are free of quinones and anthraquinones with the exception of *A. cordifolia*.Leukoanthocyanins are present only in extracts of *A. cordifolia* and *T. tetraptera*. On the other hand, gallic tannins are absent from *V. colorata* extracts. Several phytochemicals were identified in the different extracts studied. From the least rich extract to the richest in these compounds we have*O. gratissimum* (6 compounds) <*V. colorata* (7 compounds) <*T. tetraptera* and *A. cordifolia* (9 compounds).

3.2 Contents of total polyphenols, total flavonoids and Total tannins of plant extracts studied

The content of total polyphenols and total flavonoids in the plant extracts studied is summarized in Table II. The extract from the leaves of *A. cordifolia* has the highest total polyphenols content with 57.56 ± 3.23 mg/g GAE DE while the extract from the fruit of *T. tetraptera* has the lowest total polyphenols content (14.33 ± 1.45 mg/g GAEDE). In addition, the total flavonoids content of the aqueous extracts of plants studied is between 21.01 ± 1.87 mg/g QE DE and 58.10 ± 2.88 mg/g QE DE (Table III). In descending order of total flavonoid content, we have: *V. colorata*>*A. cordifolia*>*O. gratissimum*>*T. tetraptera*.Table IV shows the total tannin contents of our aqueous extracts studied, which vary from one extract to another. Thus, in ascending order of total tannin content we have: T. tetraptera (0.18 ± 0.01 mg/g TAE DE) <*V. colorata* (0.34 ± 0.01 mg/g TAE DE) < O. gratissimum (0.42 ± 0.02 mg/gTAEDE) <*A. cordifolia* (0.84 ± 0.02 mg/gTAE DE).

3.3 Hypoglycemic and antihyperglycemic activity of plant extracts studied

Figures 4; 5; 6 and 7 present the effect of aqueous extracts of the plants studied and glibenclamide (reference hypoglycemic agent) on the glycemia of normoglycemic mice. All plant extracts studied

resulted in a decrease in blood glucose in mice compared to basal blood glucose in normoglycemic mice. In addition, mice given the aqueous extracts of *A. cordifolia* at doses of 300 and 600 mg/kg BW had a similar blood glucose level to those treated with glibenclamide after 30 min. Similarly, after 120 min of experimentation, the blood glucose of mice treated with *T. tetraptera* extract at a dose of 600 mg/kg BW is identical to that of mice treated with glibenclamide. The same observation was made with *V. colorata* extracts at doses of 300 and 600 mg/kg BW after the same experimental time (120 min). For the glucose tolerance test, the blood glucose of mice treated with different extracts is similar to that of mice treated with glibenclamide at a dose of 300 and 600 mg/kgBW from 30 min of experimentation with the exception of *O. gratissimum*(figures 8; 9; 10; 11).

3.4 Antioxidant activity of plant extracts studied

The antioxidant activity of the plant extracts studied is shown in Figure 2. The inhibitory concentration 50 (IC_{50}) of these extracts compared to that of vitamin C is summarized in Table V.Among the extracts studied, that of *A. cordifolia* has the strongest antioxidant activity because it has an IC_{50} of 0.4190 ± 0.0002 mg/mL. has the highest antioxidant activity as it has an IC_{50} of 0.4190 ± 0.0002 mg/mL. This IC_{50} is less than 1 mg/mL.*V. colorata*and *O. gratissimum* extracts have IC_{50} of 1.2702 ± 0.0005 mg/mL and 1.6265 ± 0.0003 mg/mL, respectively. *T. tetraptera* extract has the lowest antioxidant activity with an IC_{50} of 2.1300 ± 0.0004 mg/mL.

3.5 Acute toxicity

Aqueous extracts did not cause any deaths at 5000 mg/kg BW during the 14 days of observation (Table VI). No evidence of toxicity was observed in extract-fed mice (Table VII). Similarly, oral route administration of the extracts to mice at a dose of 5000 mg/kg BW did not result in significant weight gain (P>0.05) compared to the control group (Figure 3). Also, no significant weight gain ((P>0.05) was observed in vital organs (kidney, liver and heart) compared to the control (Table VIII).

Table I. Phytochemical profile of extracts.

Chemical groups		*Extracts*			
		A. cordifolia	***V. colorata***	***O. gratissimum***	***T. tetraptera***
Polyphenols		**+**	**+**	**+**	**+**
Flavonoids		**+**	**+**	**+**	**+**
Leukoanthocyanins		**-**	**+**	**-**	**+**
Tanins	catechic	**+**	**-**	**-**	**+**
	gallic	**+**	**-**	**+**	**+**
Saponosides		**+**	**+**	**+**	**+**
	Dragendorf	**+**	**+**	**+**	**+**

Alkaloids	Mayer	+	+	-	+
Quinones and anthraquinones		+	-	-	-
Sterols / Terpenes		+	+	+	+

(+)=presence, (-)=absence

Table II. Total polyphenol content of aqueous extracts of the plants studied

Extracts	Plant part	Levels (mg/g GAEDE)
Alchorneacordifolia	Leaves	57,56 ± 3,23 a
Vernonia colorata	Leaves	20,24 ± 1,90 b, c
Ocimumgratissimum	Whole plant	18,90 ± 1,71 b, c
Tetrapleuratetraptera	Fruits	14,33 ± 1,45 d

These results are the mean of 3 tests ± standard deviation. The values assigned the same letters in the same column are not significantly different (P<0.05)

Table III. Total flavonoid content of aqueous extracts of the plants studied

Extracts	Plant part	Levels (mg/g QEDE)
Alchorneacordifolia	Leaves	37,76 ± 1,74 b
Vernonia colorata	Leaves	58,10 ± 2,88 a
Ocimumgratissimum	Whole plant	33,90 ± 2,05 b, c
Tetrapleuratetraptera	Fruits	21,01 ± 1,87 d

These results are the mean of 3 tests ± standard deviation. The values assigned the same letters in the same column are not significantly different (P<0.05)

Table IV. Tanin content of aqueous extracts of the plants studied

Extracts	Plant part	Level (mg/g TAEDE)
Alchorneacordifolia	Leaves	0,84 ± 0,02a
Vernonia colorata	Leaves	0,34 ± 0,01c
Ocimumgratissimum	Whole plant	0,42 ± 0,02b
Tetrapleuratetraptera	Fruits	0,18 ± 0,01d

These results are the mean of 3 tests ± standard deviation. The values assigned the same letters in the same column are not significantly different (P<0.05)

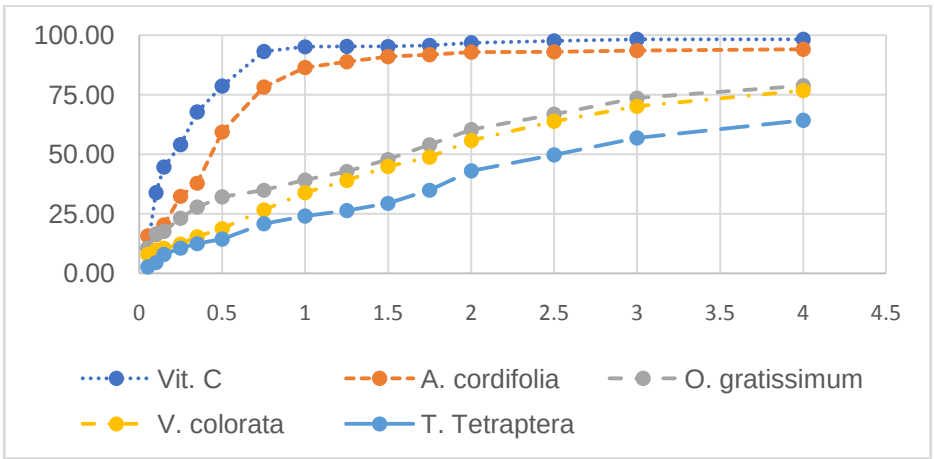

Figure 2: Antioxidant activity of aqueous extracts of the plants studied

Table V: Inhibitory concentration (IC_{50}) of aqueous extracts of the plants studied

Extracts	Plant part	IC_{50} (mg/mL)
Vitamin C		0,1325 ± 0,0003 a
Alchorneacordifolia	Leaves	0,4190 ± 0,0002 b
Vernonia colorata	Leaves	1,2702 ± 0,0005 c
Ocimumgratissimum	Whole plant	1,6265 ± 0,0003 c
Tetrapleuratetraptera	Fruits	2,13 ± 0,0004 d

These results are the mean of 3 tests ± standard deviation. The values assigned the same letters in the same column are not significantly different (P<0.05)

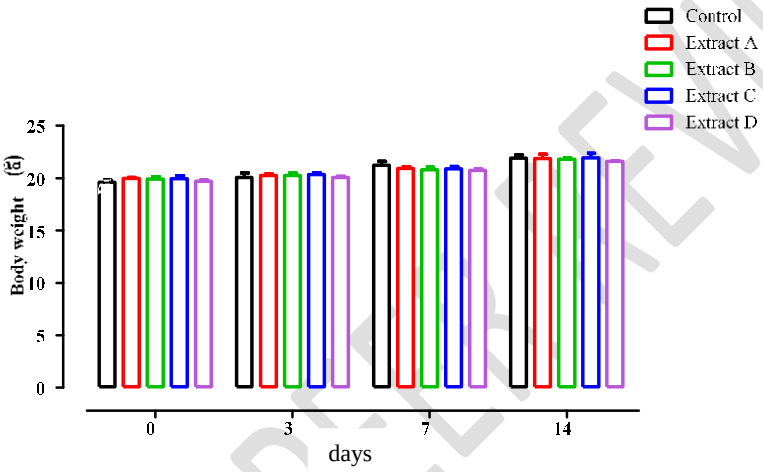

Figure 3. Evolution of body weight of control animals treated at 5000 mg/kg BW of extracts A, B, C and D in the acute toxicity study. (M ± ESM) (n=3).
A (*AlchorneaCordifolia)*, B (*Ocimumgratissimum)*, C (*TetrapleuraTetraptera*) et D (*Vernonia colorata)*

Batches	Water and Extracts	Single dose (mg/kg BW)	Number of dead rats (/3)	Rate of Mortality (%)
1	**Control (water)**	-	0	0
2	***A. cordifolia***	5000	0	0
3	***V. colorata***	5000	0	0
4	***O. gratissimum***	5000	0	0
5	***T. tetraptera***	5000	0	0

Table VI. Mortality of mice following oral administration of the 5000 mg/kg BW extract dose.

The control received only distilled water instead of extracts during the experiment.

Table VII. clinical signs observed after oral administration of the 5000 mg/kg BW extract dose

Clinicalsigns	After 14 days of observation				
	Control	*A. cordifolia*	*V. colorata*	*O. gratissimum*	*T. tetraptera*
Drowsiness	-	-	-	-	-
Stillness	-	-	-	-	-
Anorexia	-	-	-	-	-
Rapid breathing	-	-	-	-	-
Crumblingcoat	-	-	-	-	-

(-): absence of clinical signs; (+): presence of clinical signs

Table VIII. Vital organ weights of control mice treated at 5000 mg/kg BW of extracts A, B, C and D in the acute toxicity study

	Organ weights (g/100g BW)				
	Control	Extract A	Extract B	Extract C	Extract D
Kidneys	1,02 ± 0,03a	1,00 ± 0,02a	1,01 ± 0,03a	1,01 ± 0,02a	1,05 ± 0,02a
Liver	4,79 ± 0,07a	4,83 ± 0,18a	4,91 ± 0,10a	4,86 ± 0,07a	4,82 ± 0,14a
Heart	0,41 ± 0,01a	0,43 ± 0,01a	0,44 ± 0,02a	0,45 ± 0,02a	0,40 ±0,02a

These results are the mean of 3 tests ± standard deviation. The values assigned the same letters on the same line are not significantly different (P<0.05)

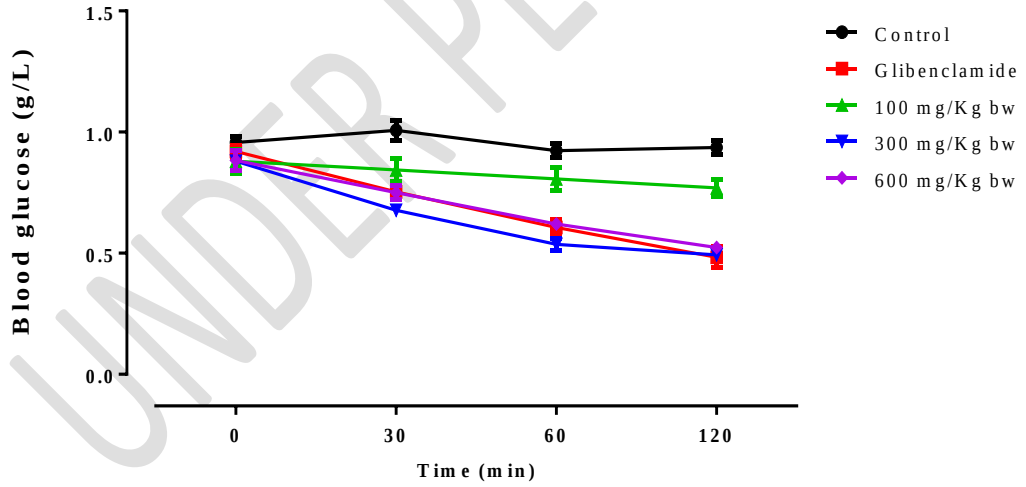

Figure 4. Effect of the aqueous extract of *A. cordifolia* and glibenclamide on basal blood glucose levels of normoglycemic mice

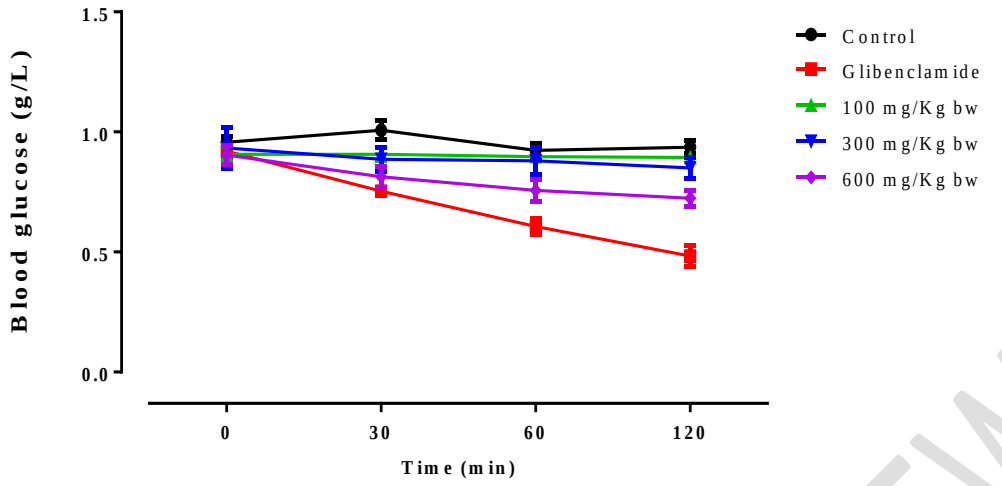

Figure 5. Effect of the aqueous extract of *O. gratissimum* and glibenclamide on basal blood glucose levels of normoglycemic mice

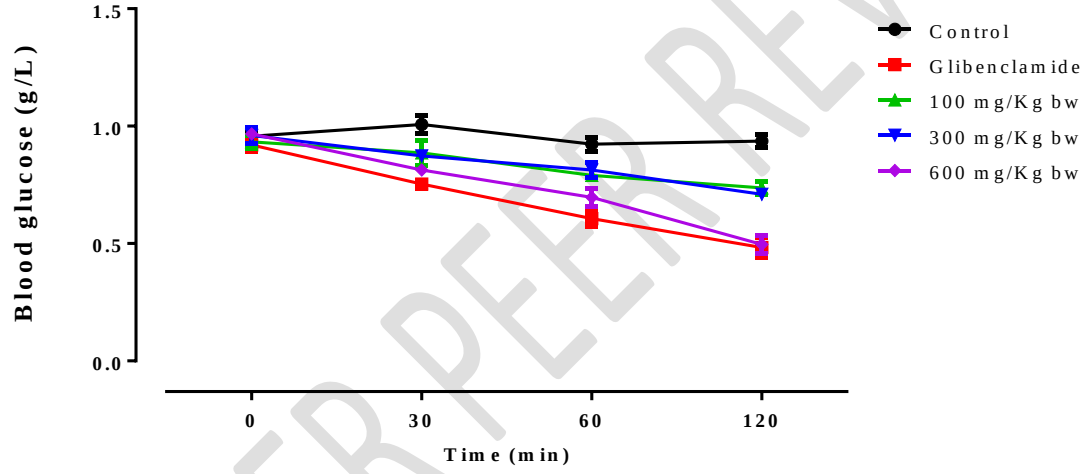

Figure 6. Effect of the aqueous extract of *T. tetraptera* and glibenclamide on basal blood glucose in normoglycemic mice.

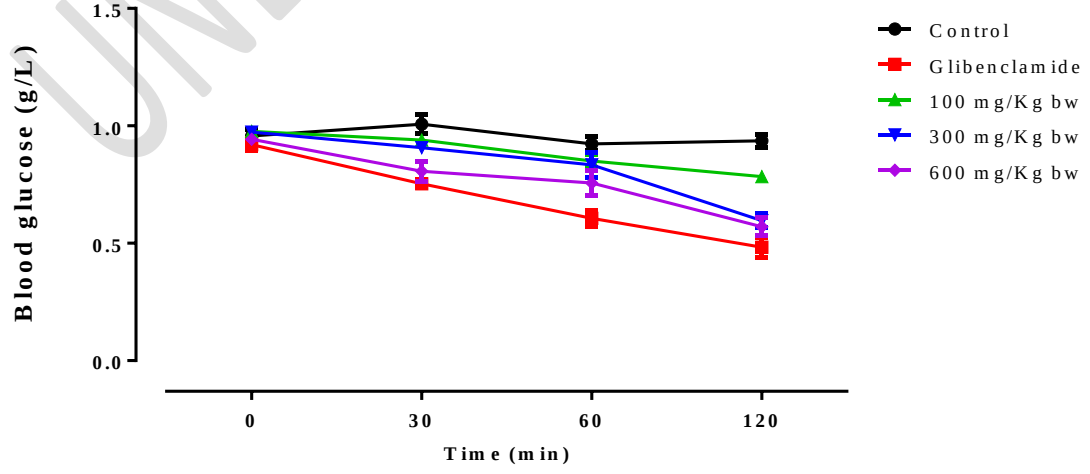

Figure 7. Effect of aqueous extract of *V. colorata* and glibenclamide on basal glucose levels of normoglycemic mice

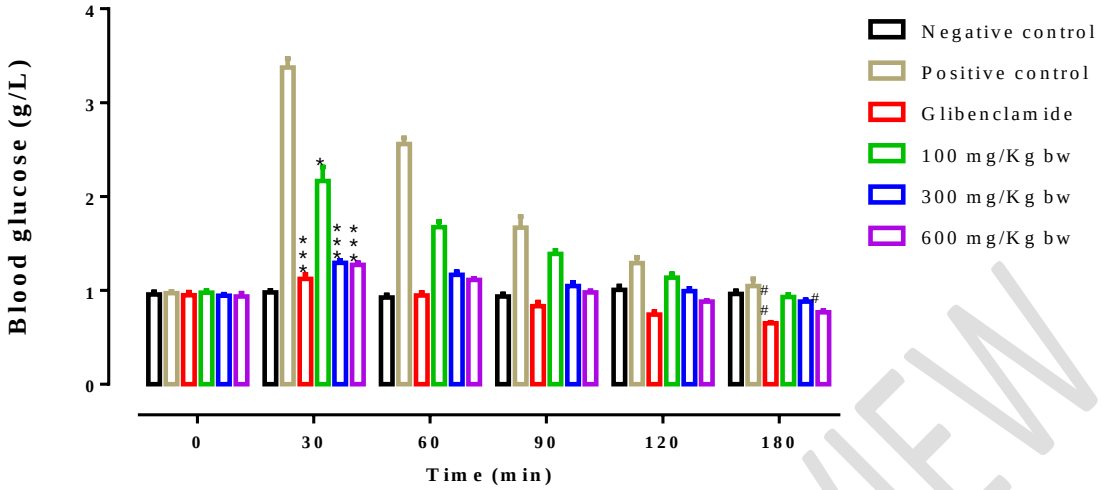

Figure 8. Effect of the aqueous extract of *A. cordifolia* and glibenclamide on glucose tolerance test mice

(M ± ESM) (n=5); (*) comparison with the positive control; (#) comparison with baseline blood glucose; *(P < 0.05), ***(P < 0.001)

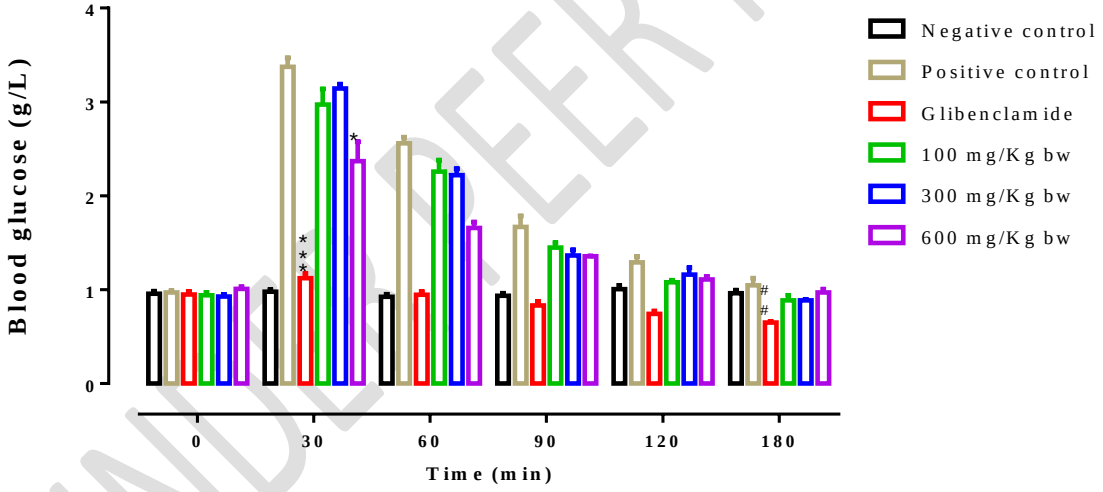

Figure 9. Effect of the aqueous extract of *O. gratissimum* and glibenclamide on glucosetolerance test mice

(M ± ESM) (n=5);**(*)** comparaison par rapport au Témoin positif ; **(#)** comparaison par rapport à la glycémie initiale ; *(P < 0,05), **(P < 0,01) ; ***(P < 0,001)

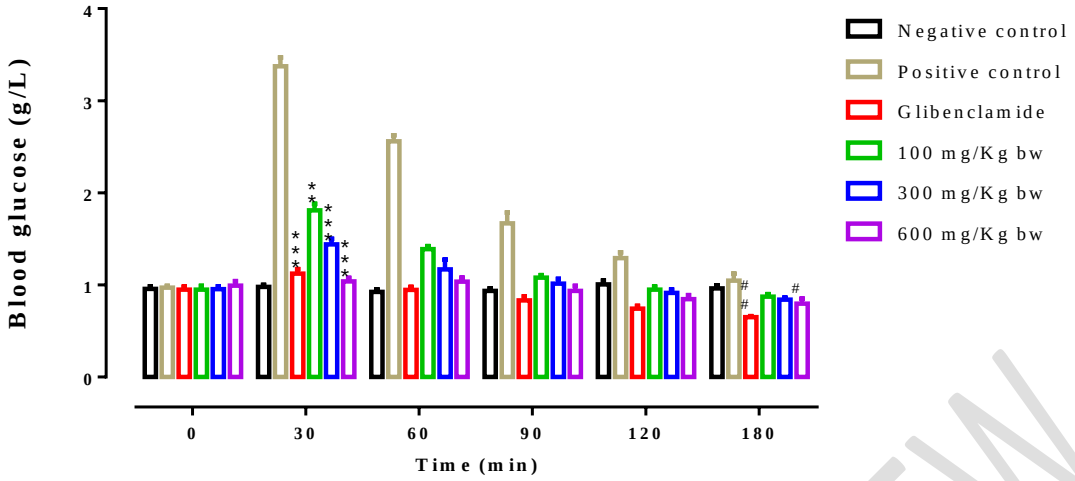

Figure 10. Effect of aqueous extract of *T. tetraptera* and glibenclamide on glucose tolerance test mice

(M ± ESM) (n=5); (*) comparison with the positive control; (#) comparison with baseline blood glucose; *(P < 0.05), **(P < 0.01); (P < 0.001)

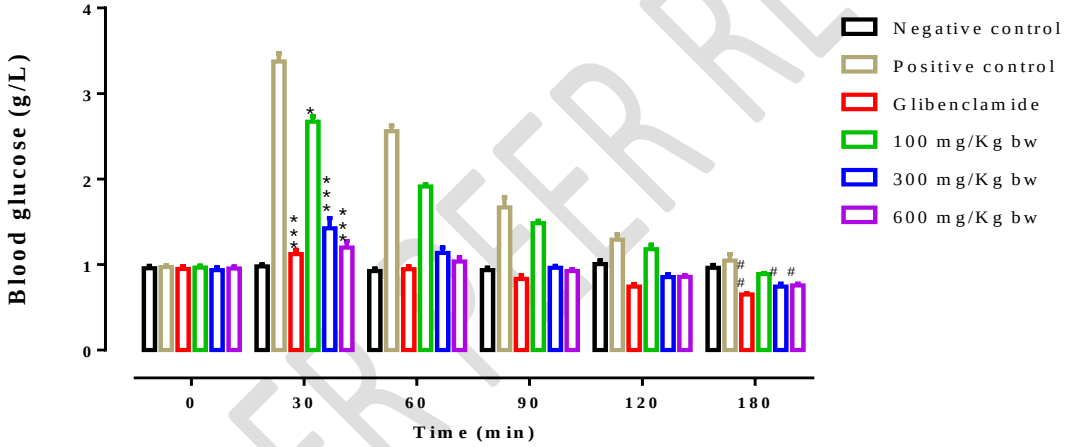

Figure 11: Effect of *V. colorata* extract and glibenclamide on blood glucose glucose tolerance test mice

(M ± ESM) (n=5); (*) comparison with the positive control; (#) comparison with baseline blood glucose; *(P < 0.05), **(P < 0.01); (P < 0.001)

4. DISCUSSION

The phytochemical profile indicates the presence of various secondary metabolites in the aqueous extracts of plants studied, namely polyphenols, flavonoids, alkaloids as well as saponosids and sterols/terpenes. Tannins are also present in all extracts except that of *V. colorata*. These results are close to those reported by Mambé et al.**[16]**. Moreover, the results of the work of Mambé et al.did not reveal the presence of anthraquinones. Similarly, those of Ogheneochuko et al.**[17]**(a), did not find flavonoids and steroids in A. cordifolia extracts. Also, the presence of polyphenols, tannins and saponosids in aqueous extracts of *V. colorata* leaves has been confirmed by the work of Sawadogo et al.**[18]** with the exception of alkaloids. The presence of polyphenols, flavonoids, tannins, alkaloids, saponins as well as sterols and terpens in *O.gratissimum*extracts is confirmed by the results of Kpètèhoto et al.**[19]** with the exception of leucoanthocyanins, as well as those obtained by N'Guessan et al.**[20]** with the absence of saponins. Also, the phytochemical composition results of the aqueous extract of *T. tetraptera* are similar to those found by Mbieleu et al.**[21]**; Larbie et al.**[22]** and Obeng et

al.**[23]**. The presence or absence of certain secondary metabolites in the same plant species studied from one author to another would be due to climatic conditions**[24]**, temperature and extraction solvents used**[25]** and extraction methods applied**[26]**; **[27]**, .

Indeed, some polyphenols, alkaloids, saponins, flavonoids and terpenoids isolated from medicinal plants are endowed with hypoglycemic power**[28]**, **[29]**. Based on work by Tang et al.**[30]**; Zhang et al.**[31]**, some alkaloids exert hypoglycaemic activity by inhibiting glucagon production. They also increase insulin production by regenerating and cleansing pancreatic β cells of free radicals.

As for saponins, they stimulate the release of insulin from the pancreas**[32]**, **[33]**, **[34]**, **[35]**. Similarly, some terpenoids exert antidiabetic activity by reducing glucose uptake and producing endogenous glucose while increasing insulin sensitivity **[36]**.

The work of Manaharan et al.**[37]** showed that phenolic compounds (phenylpropanoic acid, ferrulic acid, caffeic acid and coumarin) present in *T. tetraptera* fruits are endowed with a strong diuretic, antidiabetic, antioxidant and anti-inflammatory power. Several studies have confirmed these results such as those reported by Ojewole and Adewunmi,**[38]**; Kostova et al.**[39]** ; Gloria et al.**[40]**.

Through their anti-inflammatory action on the β cells of the pancreas, polyphenols exert a hypoglycemic effect by increasing insulin production**[41]**, **[42]**. The work conducted by Prabhakar and Doble,**[43]** showed that phenolic acids inhibited glucose absorption thus preventing hyperglycemia by performances comparable to metformin and thiazolodinedione, the main oral route hypoglycemic drug. The work of Iwai,**[44]**, Cabrera et al.**[45]** and Tadera et al.**[46]** also showed the antidiabetic activity of polyphenols, in particular flavonoids, phenolic acids and tannins by their actions on carbohydrate metabolism. Indeed, these metabolites inhibit the action of α-glucosidase and α-amylase, the key enzymes responsible for the digestion of dietary carbohydrates into glucose.

Quantitative analysis of aqueous extracts from the leaves of *A. cordifolia, V. colorata*, the whole plant of *O. gratissimum* and the fruits of *T. tetraptera* have shown that these extracts are rich in total polyphenols, total flavonoids and total tannins. These aqueous extracts also have important antioxidant capacities with an IC50 of 0.4190 ± 0.0002 mg/mL for *A. cordifolia* extract. The high antioxidant capacity of these extracts is due to the presence of these secondary metabolites and their high contents. These antioxidants, by trapping free radicals, will participate in the reduction of oxidative stress which is one of the mechanisms responsible for the development and progression of micro and macrovascular complications of diabetes. Samocha-Bonet et al.**[47]**, Hoehn et al.**[48]** and de Pérez-Matute et al.**[49]** have shown that antioxidant molecules present in plants protect against the development and complications of type 2 diabetes and also against atherosclerosis and hypertension. Antioxidants would then play a protective role against oxidative damage and insulin resistance.

Oral route administration of the extracts to mice at a dose of 5000 mg/kg bw did not result in significant weight gain (P>0.05) compared to the control group. The absence of clinical signs and mortality of animals (mice) following oral administration of the leaf and fruit extracts studied are consistent with the results obtained by several authors. Indeed, Gasting et al.**[50]** reported a lethal dose (LD50) of *A. cordifolia* leaf extracts greater than 32 g/kg bw. These results were later confirmed by those of Mahama et al.**[51]** who observed no signs of toxicity or mortality following oral administration of *A. cordifolia* extracts at a dose of 2000 mg/kg bw. The antioxidant, hepatoprotective and antimicrobial activities of *A. cordifolia* are an asset for the protection of certain organs such as the pancreas, liver, kidneys, heart, spleen, prone to tissue damage in an environment of chronic hyperglycemia**[52]**, **[53]**. Also, the work of Hounsa et al.**[54]** showed that extracts of *O. gratissimum* are not toxic orally because their administration to mice did not cause mortality or signs of toxicity. Hounsa et al., also revealed that the administration of these extracts did not cause any significant weight change (P>0.05) of the treated animals compared to the corresponding controls. Bonsou et al.**[55]** showed that the fruit of *T. tetraptera* was safe at a single dose of 5000mg/kg bw. Also, according to the results of Sawadogo et al.**[18]** oral administration of *V. colorata* at a dose of 5000 mg/kg bw does not expose the consumer to toxicity risks. According to OECD Test Guideline 423 for chemicals, the leaf and fruit extracts studied have a lethal dose (LD50) greater than 5000 mg/kg bw**[56]**. The absence of mortality following the administration of these extracts orally allows these

extracts to be classified in category 5 according to the global harmonization system because all extracts have a lethal dose between 5000 and 15000 mg/kg bw according to the Hodge and Sterner scale**[15]**. These extracts could therefore be considered to be low or non-toxic in a single dose via the oral route**[57]**.

5. CONCLUSION

This study showed that the leaf extracts of *A. cordifolia* and *V. colorata*, the whole plant of *O. gratissimum* and the fruits of *T. tetraptera* studied contain several chemical groups namely total polyphenols, total flavonoids, alkaloids, total tannins, saponins and sterols and polyterpens with important pharmacological effects. These aqueous extracts including that of *A. cordifolia* have a strong antioxidant capacity as well as a good hypoglycemic and antihyperglycemic activity at a dose of 300 mg/kg bw. In addition, these compounds are non-toxic y oral route up to a dose greater than 5000 mg/kg bw (LD50 > 5000 mg/kg bw). Therefore, oral route administration of these extracts does not present a danger to the consumer. This justifies their use in traditional medicine for the treatment of various cardiovascular diseases including diabetes. It would be interesting to conduct an in-depth study of the subacute toxicity of these extracts in order to evaluate their effect on the noble organs of the heart, liver, kidneys and lungs.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

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