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Phytochemical Screening, Antioxidant Activity and Cytotoxicity of four Medicinal Plants for Antidiabetic Purposes Used in the Ivorian Pharmacopoeia

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Aims: This study aims to investigate the phytochemical profile, antioxidant activity and cytotoxicity of aqueous extracts from four plants used in the Yamoussoukro district (Côte d'Ivoire) for the treatment of diabetes.

Methodology: Secondary metabolites of four plants (*Alchornea cordifolia, Ocimum gratissimum, Tetrapleura tetraptera and Vernonia colorata*) were carried out by phytochemical screening using appropriate reagents. Polyphenol and tannin contents were determined using the Folin- ciocalteu colorimetric method. The antioxidant activity of the various extracts was then assessed in vitro using the DPPH (1,1-diphenyl-2-picryl-hydrazyl) assay. Acute toxicity was also assessed by administering extracts orally to mice in single increasing doses. Hypoglycemic and antihyperglycemic activities were determined by monitoring blood glucose levels in mice after administration of the extracts.

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

Conclusion and Outlook: These results show that the plant extracts studied contain several secondary metabolites responsible for their good antioxidant capacities. What's more, the plants studied have good anti-diabetic activity and are non-toxic by the oral route. This could justify their use in traditional medicine to combat diabetes.

Keywords: Medicinal plants; phytochemistry; toxicity; antioxidant; antidiabetic properties.

1. INTRODUCTION

Diabetes is a disease of endocrine and metabolic disorders characterized by chronic hyperglycemia. It is caused either by a disturbance in insulin secretion or function, or both [1]. It increases the risk of cardiovascular disease complications [1,2] and poses a real public health problem. Indeed, diabetes represents the third most common chronic disease after cancer and cardiovascular disease. According to the International Diabete Federation (IDF), in 2017, there were 451 million diabetics worldwide. This figure rose to 536 million in 2021 and is expected to reach 783 million by 2045 [3,4].

Despite the presence of anti-diabetic drugs on the pharmaceutical market, herbal treatment of diabetes is practised by over 80% of the rural population. For centuries, plants have been considered a fundamental source of medicines for health care. In developing countries, in general, medicinal plants are used to treat diabetes to offset the high cost and accessibility of conventional drugs for the low-income population [5]. Today, 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 anti-diabetic properties [7] and without notable side effects [8].

Faced with the high cost of modern medicines and the side effects of their prolonged use, the Wold Health Organisation (WHO), in its resolution AFR/RC50/R3 of August 31 2000, encourages African countries to develop regional strategies on traditional medicine, in order to undertake research on medicinal plants and promote their optimal use in healthcare delivery systems. These plants include *Alchornea cordifolia, Ocimum gratissimum, Tetrapleura tetraptera* and *Vernonia colorata,* which are used in the Yamoussoukro district to treat diabetes.

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

2. MATERIALS AND METHODS

2.1 Plant Materials

The plant material consisted of the leaves of Alchornea cordifolia and Vernonia colorata, the whole plant of Ocimum gratissimum collected in Djahakro and Kami, villages located in the Yamoussoukro locality (Côte d'Ivoire) and the fruits of Tetrapleura tetraptera collected in Sikensi in the Agnéby-Tiassa region. These plant species were identified by botanist N'GUESSAN Amani in accordance with the herbaria avalables at the Higher School of Agronomy of the National Polytechnic Institute Houphouët Boigny of Yamoussoukro.

After harvest, the plant material sent to the laboratory was dried at room temperature for 14 days, and then crushed and stored for further experiments.

2.1.1 Preparation of plants extracts

Extracts were obtained from dried sample powders using the method described by Bidié et al. [9]. 100g of each powder was mixed with 1L of distilled water. The mixture was heated at reflux for 15 min. The mixture was then cooled and filtered through Whatman paper. The filtrate obtained was dried at 55°C to obtain the dry extract.

2.2 Animal Materials

Mice of the Mus musculus species, Swiss strain, were used for the various in vivo experiments. They were caged in groups of 5 with a 12/12 h light/dark cycle and an ambient temperature of 28 ± 2°C. The animals were fed pellets from FACI (Ivorian Compound Feed Manufacturing Company) and given tap water without interruption.

Female mice were used for the acute oral toxicity test. They were nulliparous and non-pregnant. They were 8 weeks old and weighed between 19 and 21 g.

Male mice were used for the hypoglycemic and antihyperglycemic activity tests. They ranged in age from 9 to 10 weeks and body weight from 25 to 28 g.

Male mices were used for testing hypoglycemic and antihyperglycemic activities. Their age ranging from 9 to 10 weeks and their BW from 25 to 28 g.

2.3 Identification of Phytochemical Groups of Extracts

Different families of secondary metabolites such as polyphenols, flavonoids, leuco-anthocyanins, tannins, saponosides, alkaloids, quinones, sterols/terpenes, have been highlighted in the extracts using the method described by Bagré et al. [10].

2.4 Determination of Total Polyphenols Content

Determination of total polyphenols was carried out by the colorimetric method using Folin-Ciocalteu reagent according to the method described by Wood et al, [11]. To a test tube containing 30 µL of extract was added 2.5 mL of Folin-Ciocalteu reagent diluted 1/10. The mixture was left in the dark for 2 minutes at room temperature (30±2°C). Next, 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 rapidly cooled. Absorbance was measured using 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. Total polyphenol content was expressed in milligrams per liter of extract gallic acid equivalent (mg/L GAE).

2.5 Determination of Total Flavonoids Content

Total flavonoids were determined in accordance with Marinova et al, [12]. In a 25 mL flask, 0.75 mL sodium nitrite ($NaNO_2$) 5% (w/v) was added to 2.5 mL extract. The mixture was supplemented with 0.75 mL 10% (w/v) aluminum chloride (AlCl3), then incubated for 6 minutes in the dark. After incubation, 5 mL of sodium hydroxide (NaOH 1N) was added and the volume made up to 25 mL. After vigorous agitation of the mixture, absorbance was measured with a UV-visible spectrophotometer at wavelength ʎ= 510 nm. Flavonoid content was expressed in mg QE (Quercetin Equivalent) per liter of extract. A calibration line was run with quercetin at different concentrations.

2.6 Determination of Total Tannins Content

Total tannins were determined using the colorimetric method with Folin Ciocalteu reagent as described by Chandran and Indira [13]. 100

µL of extract was added to a test tube containing 7.5 mL distilled water and 0.5 mL Folin Ciocalteu reagent. Next, 1 mL of 35% Na_2CO_3 was added. The volume is made up to 10 mL by adding 900 µL distilled water. The reaction mixture was incubated for 30 min at laboratory temperature (25-30°C). Absorbances were read with a UV/visible spectrophotometer at 700 nm against distilled water used as a blank. Contents were expressed in micrograms of tannic acid equivalent per liter of extract (mg EAT/L).

2.7 Evaluation of Antioxidant Activity (AAO) of Extracts by DPPH

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

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

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

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 carried out in a sequential procedure, using 3 mice at each stage, in accordance with OECD [15] guideline 423. A fixed dose of the extract was administered orally to a group of animals. The absence or manifestation of extract-related mortality in a group dosed at a given step 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 body weight (BW), 15 female mice divided into 5 batches of 3 were used for the 5000 mg/kg BW dose. The first batch received distilled water only (control batch). Each of the other four batches received an oral extract corresponding to the 5000 mg/kg dose of BW. The animals were then observed for 14 days.

2.9 Hypoglycemic Activity in Normoglycemic Animals

Animals were fasted for 14 hours without water deprivation. Basal blood glucose levels were then measured before administration of the corresponding extracts in each batch. Blood glucose levels were checked at 30, 60 and 120 minutes. Percentage changes in blood glucose levels were calculated at the various 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 levels were measured prior to administration of the corresponding extracts for each batch. The animals received anhydrous glucose (4mg/Kg of glucose BW) followed by the various test substances at virtually the same time (approx. 1 minute apart). Blood glucose levels were monitored every 30 minutes for 180 min. Percentage changes in blood glucose levels were calculated at the various glucose measurement times.

2.11 Statistical Analysis

Statistical analysis was performed using a one-way analysis of variance (ANOVA) for all data (mean of each parameter measured). The various values obtained were expressed as the mean followed by the standard error of the mean (M±ESM). Comparisons of means were made using 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 phytochemical compounds of the plant extracts studied are summarized in Table I. Phenolic compounds, saponosides and sterols/terpenes are present in all the extracts studied. In addition, all extracts were free of

quinones and anthraquinones, with the exception of *A. cordifolia*. Leuco-anthocyanins are present only in extracts from *A. cordifolia* and *T. tetraptera*. Gallic tannins, on the other hand, were absent from *V. colorata* extracts. Several phytochemical compounds were identified in the various extracts studied. From the extract with the lowest to the highest content of 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 total polyphenols and total flavonoids content in the plant extracts studied is summarized in Table 2. The extract from *A. cordifolia* leaves had the highest total polyphenols content with 57.56 ± 3.23 mg/g GAE while the extract from *T. tetraptera* fruit had the lowest total polyphenols content (14.33 ± 1.45 mg/g GAE). The total flavonoids content of the aqueous plant extracts studied ranged from 21.01 ± 1.87 mg/g QE and 58.10 ± 2.88 mg/g QE (Table 3). In descending order of total flavonoid content, we have: *V. colorata* > *A. cordifolia* > *O. gratissimum* > *T. tetraptera*. Table 4 shows the total tannin contents of our aqueous extracts studied, which varies from one extract to another. In ascending order of total tannin content, we have: *T. tetraptera* (0.18 ± 0.01 mg/g TAE) < *V. colorata* (0.34 ± 0.01 mg/g TAE) < *O. gratissimum* (0.42 ± 0.02 mg/g TAE) < *A. cordifolia* (0.84 ± 0.02 mg/g TAE).

3.3 Antioxidant Activity of Plant Extracts Studied

The antioxidant activity of the plant extracts studied is shown in Fig. 2. The inhibitory concentration 50 (IC_{50}) of these extracts compared to that of vitamin C is summarized in Table 5. 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.

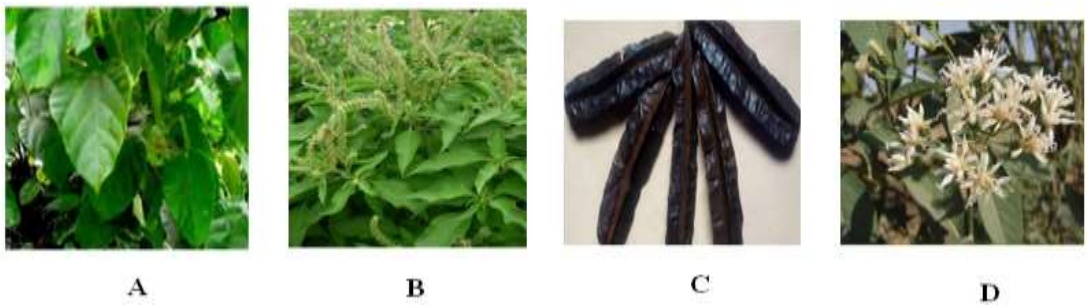

Fig. 1. A (*Alchornea Cordifolia*), B (*Ocimum gratissimum*), C (*Tetrapleura tetraptera*) and D (*Vernonia colorata*)

Table 1. Phytochemical profile of extracts

Chemical groups		Extracts			
		A. cordifolia	***V. colorata***	***O. gratissimum***	***T. tetraptera***
Polyphenols		+	+	+	+
Flavonoids		+	+	+	+
Leukoanthocyanins		-	+	-	+
Tanins	catechic	+	-	-	+
	gallic	+	-	+	+
Saponosides		+	+	+	+
Alkaloids	Dragendorf	+	+	+	+
	Mayer	+	+	-	+
Quinones and anthraquinones		+	-	-	-
Sterols / Terpenes		+	+	+	+

(+) = presence, (-) = absence

Table 2. Total polyphenol content of aqueous extracts from the plants studied

Extracts	Plant part	Levels (mg/g GAE)
Alchornea cordifolia	Leaves	57,56 ± 3,23 a
Vernonia colorata	Leaves	20,24 ± 1,90 b, c
Ocimum gratissimum	Whole plant	18,90 ± 1,71 b, c
Tetrapleura tetraptera	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 3. Total flavonoid content of aqueous extracts from the plants studied

Extracts	Plant part	Levels (mg/g QE)
Alchornea cordifolia	Leaves	37,76 ± 1,74 b
Vernonia colorata	Leaves	58,10 ± 2,88 a
Ocimum gratissimum	Whole plant	33,90 ± 2,05 b, c
Tetrapleura tetraptera	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 4. Tanin content of aqueous extracts from the plants studied

Extracts	Plant part	Levels (mg/g TAE)
Alchornea cordifolia	Leaves	0,84 ± 0,02a
Vernonia colorata	Leaves	0,34 ± 0,01c
Ocimum gratissimum	Whole plant	0,42 ± 0,02b
Tetrapleura tetraptera	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)

Table 5. Inhibitory concentration (IC_{50}) of aqueous extracts from the plants studied

Extracts	Plant part	IC_{50} (mg/mL)
Vitamin C		0,1325 ± 0,0003 a
Alchornea cordifolia	Leaves	0,4190 ± 0,0002 b
Vernonia colorata	Leaves	1,2702 ± 0,0005 c
Ocimum gratissimum	Whole plant	1,6265 ± 0,0003 c
Tetrapleura tetraptera	Fruits	2,1300 ± 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)

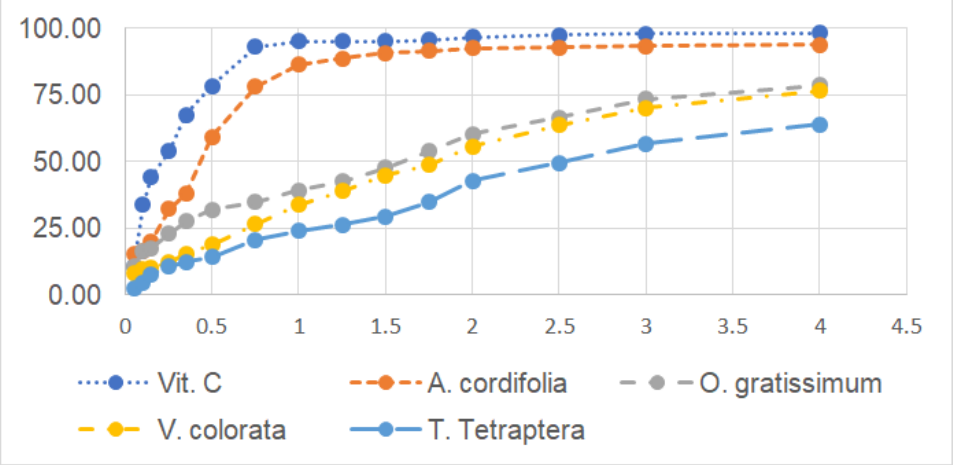

Fig. 2. Antioxidant activity of the aqueous extracts of the plants studied

3.4 Hypoglycemic and Antihyperglycemic Activity of Plant Extracts Studied

Fig. 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/kg BW from 30 min of experimentation with the exception of *O. gratissimum* (Fig 8; 9; 10; 11).

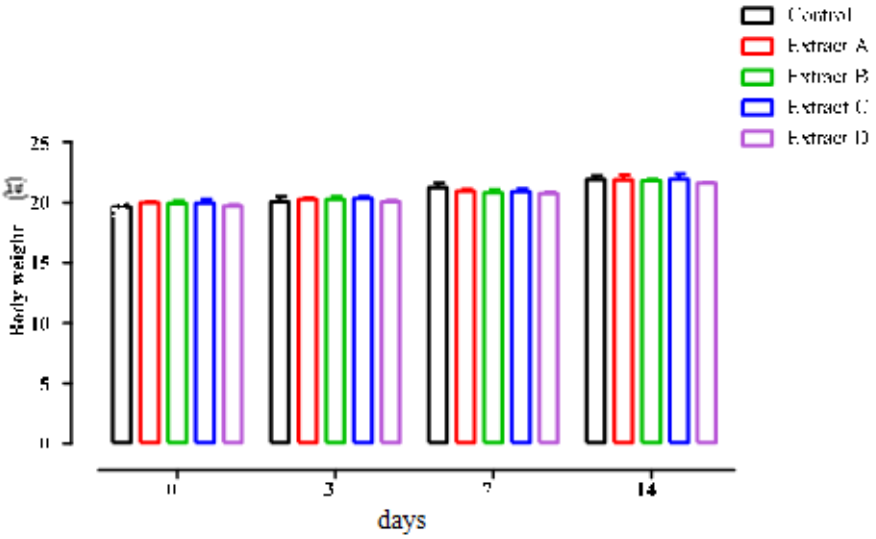

Fig. 3. Changes in body weight of control and 5000 mg/kg BW-treated animals of extracts A, B, C and D in the acute toxicity study. (M ± ESM) (n=3)

A (Alchornea Cordifolia), B (Ocimum gratissimum), C (Tetrapleura Tetraptera) et D (Vernonia colorata)

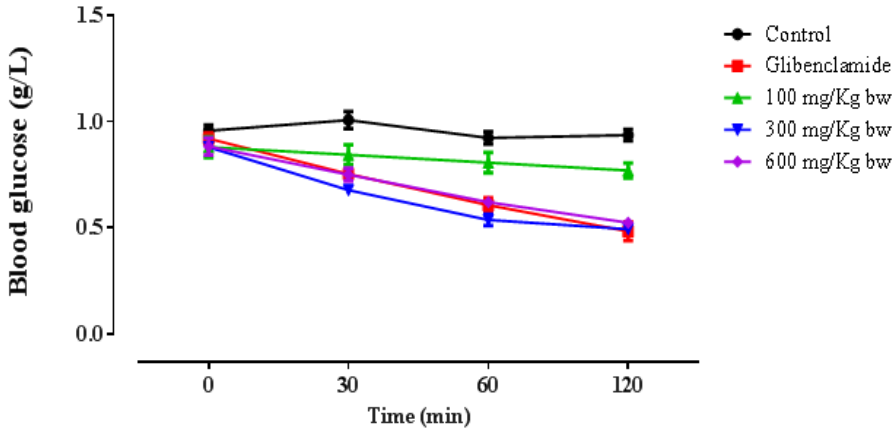

Fig. 4. Effect of *A. cordifolia* aqueous extract and glibenclamide on basal blood glucose levels of normoglycemic mice

3.5 Acute Toxicity

Aqueous extracts did not cause any deaths at 5000 mg/kg BW during the 14 days of observation (Table 6). No evidence of toxicity was observed in extract-fed mice (Table 7). 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 (Fig. 3). Also, no significant weight gain ((P>0.05) was observed in vital organs (kidney, liver and heart) compared to the control (Table 8).

Table 6. Mortality of mice after oral administration of 5000 mg/kg BW extract

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

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

Table 7. Clinical signs observed after oral administration of the 5000 mg/kg BW extract dose

Clinical signs	After 14 days of observation				
	Control	*A. cordifolia*	*V. colorata*	*O. gratissimum*	*T. tetraptera*
Drowsiness	-	-	-	-	-
Stillness	-	-	-	-	-
Anorexia	-	-	-	-	-
Rapid breathing	-	-	-	-	-
Crumbling coat	-	-	-	-	-

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

Table 8. Vital organ weights of control and 5000 mg/kg BW-treated mice from extracts A, B, C and D in 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)

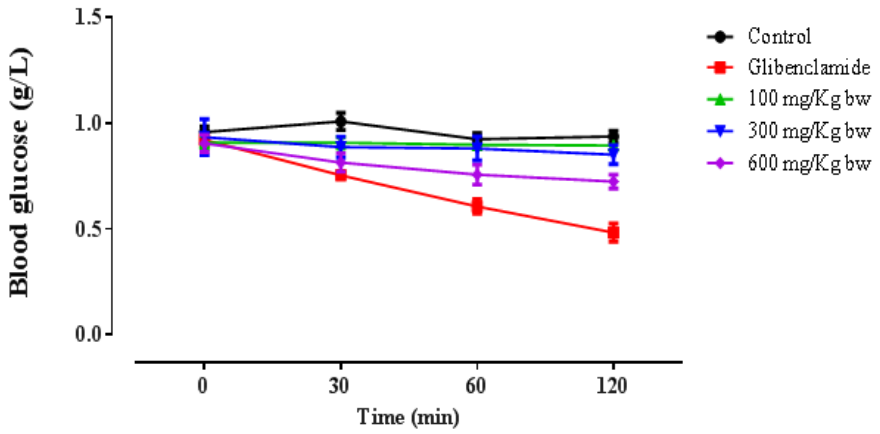

Fig. 5. Effect of *O. gratissimum* aqueous extract and glibenclamide on basal blood glucose levels of normoglycemic mice

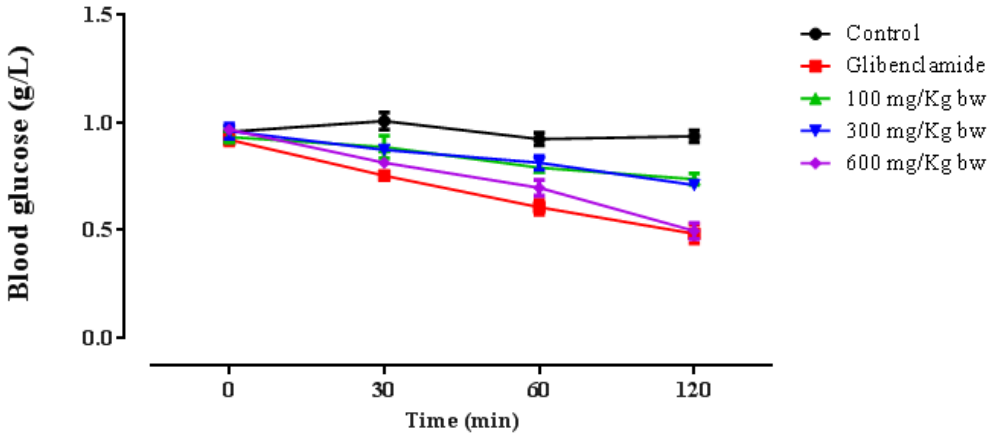

Fig. 6. Effect of *T. tetraptera* aqueous extract and glibenclamide on basal blood glucose in normoglycemic mice

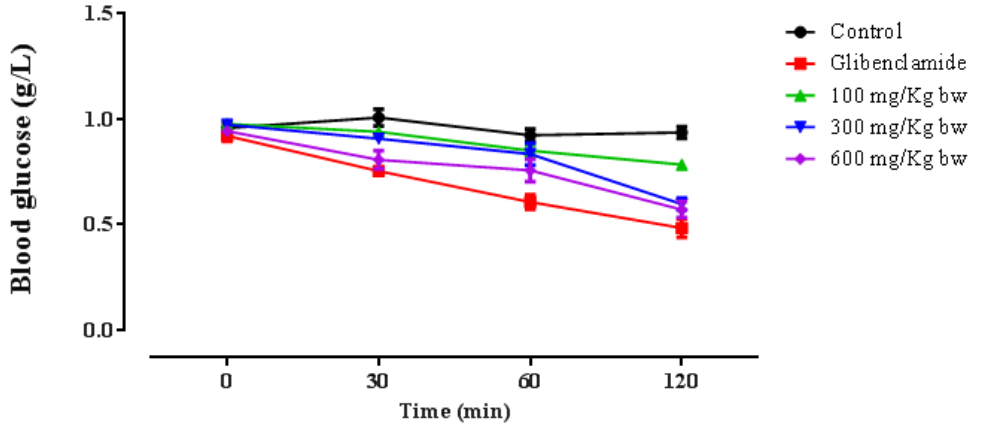

Fig. 7. Effect of *V. colorata* aqueous extract and glibenclamide on basal glucose levels of normoglycemic mice

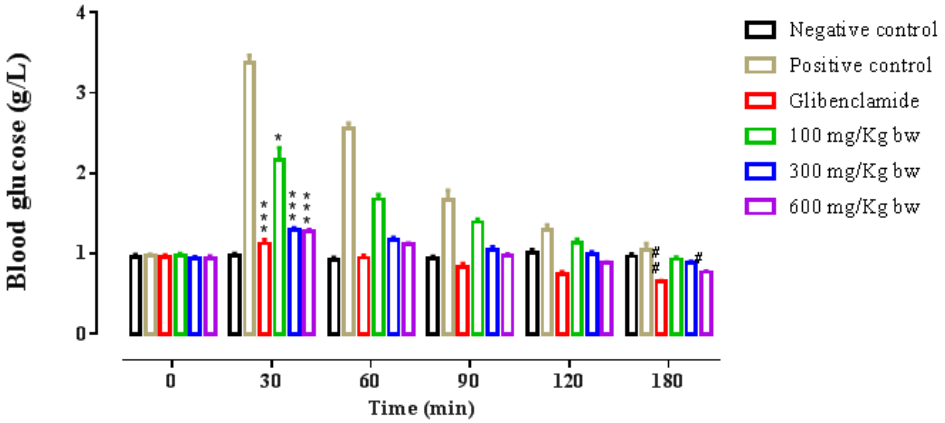

Fig. 8. Effect of *A. cordifolia* aqueous extract and glibenclamide on blood glucose levels in mice subjected to glucose tolerance test

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

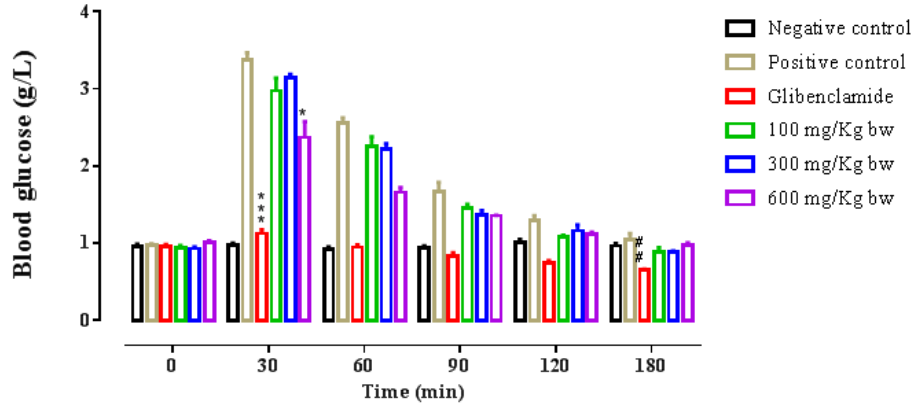

Fig. 9. Effect of *O. gratissimum* aqueous extract and glibenclamide on blood glucose levels in mice subjected to glucose tolerance test

*(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)*

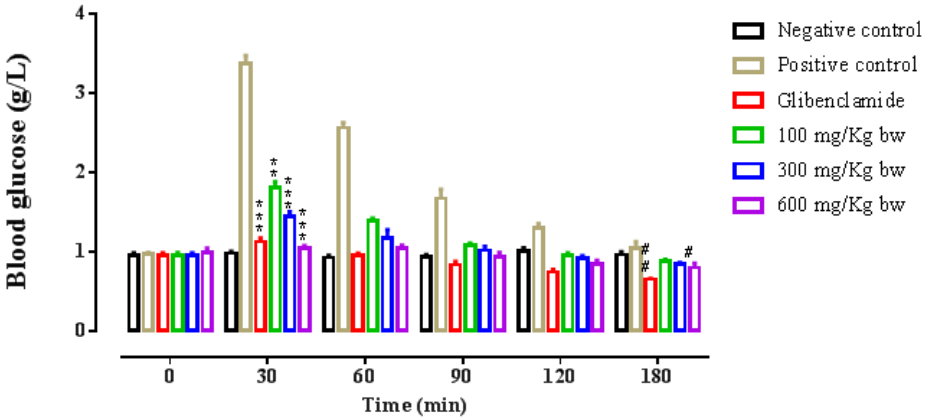

Fig. 10. Effect of *T. tetraptera* aqueous extract and glibenclamide on blood glucose levels in mice subjected to glucose tolerance test

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

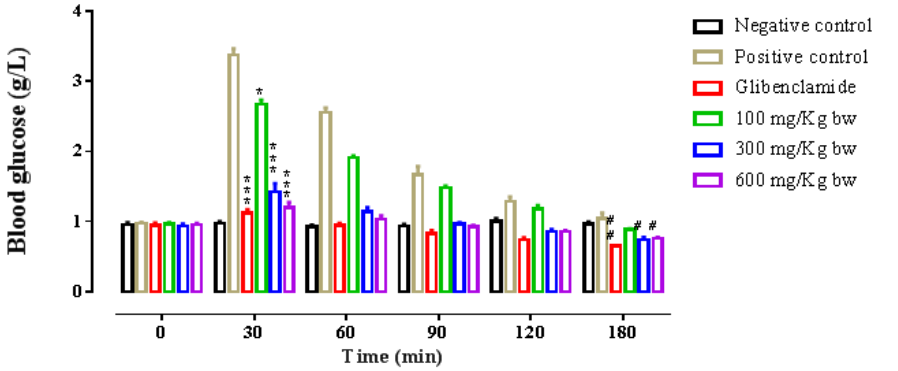

Fig. 11. Effect of *V. colorata* aqueous extract and glibenclamide on blood glucose levels in mice subjected to glucose tolerance test

*(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 the plants studied, namely polyphenols, flavonoids, alkaloids as well as saponosids and sterols/terpenes. Tannins were 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 by Mambé et al. did not reveal the presence of anthraquinones. Similarly, Ogheneochuko et al. [17] found no flavonoids or steroids in *A. cordifolia* extracts. Also, the presence of polyphenols, tannins and saponosides in aqueous extracts of *V. colorata* leaves was 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 terpenes 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 results of the phytochemical composition of the *T. tetraptera* aqueous extract 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 climatiques conditions [24], the temperature and extraction solvents used [25] and the extraction methods applied [26,27].

Indeed, some polyphenols, alkaloids, saponins, flavonoids and terpenoids isolated from medicinal plants are endowed with hypoglycemic power [28,29]. According to work by Tang et al, [30] ; Zhang et al. [31], some alkaloids exert hypoglycemic 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 insulin release 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] has shown that the phenolic compounds (phenylpropanoic acid, ferrulic acid, caffeic acid and coumarin) present in *T. tetraptera* fruits have strong diuretic, antidiabetic, antioxidant and anti-inflammatory properties. Several studies have confirmed these results, such as those reported by Kuate et al., [38] ; Kostova et al., [39] Gloria et al. [40].

Through their anti-inflammatory action on β cells of the pancreas, polyphenols exert a hypoglycemic effect by increasing insulin production [41,42]. Work by Prabhakar and Doble, [43] showed that phenolic acids inhibited glucose absorption, thus preventing hyperglycemia with performance comparable to that of metformin and thiazolodinedione, the main oral hypoglycemic drugs. The work of Aryaeian et al., [44], Cao et al, [45] and Rasines-Perea et Tei [46] has also demonstrated the anti-diabetic activity of polyphenols, in particular flavonoids, phenolic acids and tannins, through their actions on carbohydrate metabolism. Indeed, these metabolites inhibit the action of α-glucosidase and α-amylase, the key enzymes responsible for digesting dietary carbohydrates into glucose.

Quantitative analyses of aqueous extracts from the leaves of *A. cordifolia, V. colorata*, the whole plant of *O. gratissimum* and the fruits of *T. tetraptera* showed that these extracts are rich in total polyphenols, total flavonoids and total tannins. These aqueous extracts also display high antioxidant capacities, with an IC50 of 0.4190 ± 0.0002 mg/mL for the *A. cordifolia* extract. The high antioxidant capacity of these extracts is due to the presence of these secondary metabolites and their high content. By scavenging free radicals, these antioxidants will help reduce oxidative stress, one of the mechanisms responsible for the development and progression of the micro- and macrovascular complications of diabetes. Somacha-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, as well as atherosclerosis and hypertension. Antioxidants thus play a protective role against oxidative damage and insulin resistance.

Oral administration of the extracts to mice at a dose of 5000 mg/kg BW did not result in any significant weight gain ($P>0.05$) compared with the control batch. The absence of clinical signs and mortality of the animals (mice) following oral administration of the leaf and fruit extracts studied are in line with the results obtained by several authors. Indeed, Gasting et al. [50] reported a lethal dose (LD50) of *A. cordifolia* leaf extracts in excess of 32 g/kg BW. These results were later confirmed by 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 CP. The antioxidant, hepato-protective and antimicrobial activities of *A. cordifolia* leaves are an asset for the protection of certain organs such as the pancreas, liver, kidneys, heart and spleen, which are subject to tissue damage in an environment of chronic hyperglycemia [52,53]. Also, the work of Hounsa et al. [54] showed that *O. gratissimum* extracts are not toxic by the oral route, as their administration to mice produced no mortality or signs of toxicity. Hounsa et al. also revealed that administration of these extracts caused no significant weight variation (P>0.05) in treated animals compared with corresponding controls. Bonsou et al. [55] showed that the fruit of *Tetrapleura tetraptera* was safe at a single dose of 5000mg/kg body weight. Also, according to the results of Sawadogo et al. [18] oral administration of *V. colorata* at a dose of 5000 mg/kg body weight does not expose the consumer to toxicity risks. According to OECD guideline 423 for chemical testing, the leaf and fruit extracts studied have a lethal dose (LD50) greater than 5000 mg/kg CP [56]. The absence of mortality following oral administration of these extracts enables them to be classified in category 5 under the global harmonization system, since all extracts have a lethal dose of between 5000 and 15000 mg/kg BW according to the Hodge and Sterner scale [15]. These extracts could therefore be considered low or non-toxic in single doses 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* contained several chemical groups namely total polyphenols, total flavonoids, alkaloids, total tannins, saponins and sterols and polyterpens with important pharmacological effects. These aqueous extracts in particular 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. What's more, these compounds are non-toxic by oral route up to doses exceeding 5000 mg/kg BW (LD50 > 5000 mg/kg BW). Consequently, oral administration of these extracts presents no 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 into the subacute toxicity of these extracts with a view to assessing their effect on the noble organs of heart, liver, kidneys and lungs.

CONSENT

It is not applicable.

ETHICAL APPROVAL

Animal Ethic committee approval has been collected and preserved by the author(s)

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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