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Original Article

PHYTOCHEMICAL SCREENING, ANTIOXIDANT AND HYPOGLYCEMIC ACTIVITY OF *COCCOLOBA UVIFERA* LEAVES AND *WALTHERIA INDICA* ROOTS EXTRACTS

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ABSTRACT

Objective: *Coccoloba uvifera* (Polygonaceae) and *Watheria indica* (Sterculiaceae) are two medicinal plants used in Togolese traditional medicine to treat diabetes mellitus. The present study was designed to evaluate their antioxidant properties and hypoglycemic activity.

Methods: After phytochemical screening, hydro alcoholic leaves extracts of *Coccoloba uvifera* and the hydro alcoholic roots extract of *Watheria indica were evaluated* on blood glucose levels in fasting normal and glucose loaded hyper glycemic rats (oral glucose tolerance test). Antioxydant activities were performed using AAPH (2, 2'-Azobis 2 Amidino propane Dihydrochloride test and nitric oxide radical scavenging activity).

Results: Phytochemical tests revealed the presence of flavonoids, total phenols and tanin in the hydro alcoholic extracts of *C. uvifera* and *W. indica*. The hypoglycemic activity measured after oral administration of glucose (2 g/kg) revealed that W. indica leaves and C. uvifera roots induced a significant reduction of hyperglycemia in treated group compared to control group. There was a significant reduction in the hyperglycemic peak with W. indica leaves extract at doses of 250 mg/kg ($p<0.05$) and 500 mg/kg ($p<0.001$). Moreover, the administration of extracts (at dose of 500 mg/kg) in fasted rats did not show any significant decrease in basal blood glucose level compared to the control. Hydro alcoholic extracts inhibit hemolysis of erythrocytes induced by AAPH dose-dependent manner and have an antioxidant power comparable to that of the Butylated Hydroxy Toluene (reference drug). The extracts inhibit the production of nitric oxide and possess reducing power.

Conclusion: The results of this study support the use of these plants in the treatment of diabetes in Togolese traditional medicine.

Keywords: hypoglycemia, Oral glucose tolerance test, Antioxidant, *Coccoloba uvifera*, *Waltheria indica.*

INTRODUCTION

Diabetes is a disease characterized by chronic hyperglycemia, with fasting blood glucose higher than 1.26 g/l (7 mM/l). This high blood glucose can be caused by a defect in insulin secretion, action, or both combined anomalies. We have Insulin-Dependent Diabetes (IDDM) or type 1 which usually occurs before the age of 20 years and represents 10 to 15% of diabetes and Non-Insulin-Dependent Diabetes (NIDDM) or type 2 which occurs often after the age of 50 years and represents 85 to 90% of diabetes [1]. Because of its prevalence, mainly due to the aging population and lifestyle (sedentary lifestyle and eating habits), non-insulin dependent diabetes is a major public health problem. Its prevalence increases sharply and quickly in all countries (Wild *et al.*, 2004)[2], confirming that apart a genetic. , the disease would have one or more environmental factors. The World Health Organization (WHO) suggests a pandemic with numbers ranging from 366 million patients in 2011 to 552, million in 2030 (IDF 2011) [3]. In Togo, 2.6% of the Togolese population would be affected by the disease [4].

Current treatments are mainly represented by insulin and oral hypoglycemic agents (sulfonylureas, biguanides, alpha-glucosidase inhibitors) are intended to treat, not to cure the disease. Thus there is a renewed interest for alternative care solutions such as the use of plants. By their phytochemical composition, plants constitute important sources of natural compounds involved in the treatment of various diseases [5]. Among these molecules include antioxidants such as flavonoids, mainly were known for their modulatory properties of some enzymes activities, their vascular-protective effect [6, 7], their anti-inflammatory activities [8] and as antidiabetic drugs [9]. In the specific case of diabetes and other pharmacological activity on the Togolese flora [10, 11], two types of plant substances seem interesting: those who act as hypoglycemic and those who act as antioxidants knowing that hyperglycemia can be characterized by a high oxidative stress [12, 13].

The objective of this study is to evaluate the antioxidant properties and the hypoglycemic activity of two plants of the Togolese flora *Coccoloba uvifera* (polygonaceae) and *Waltheria indica* (sterculiaceae) used in the treatment of diabetes in traditional medicine.

MATERIALS AND METHODS

Animals

Sprague-Dawley rats (150–250 g) were used in this study. They were housed in standard environmental conditions (temperature 28–30 °C, relative humidity 40–45%, and a 12t/12 h light-dark cycle) and fed with standard rat diet and water *ad libitum*. Prior to drug administration, animals were fasted for 16 h with free access to water. They were deprived of both food and water during the experiment. Principles of laboratory animal care as described in the European Community guidelines were followed (Official Journal of European Union L197 vol. 50, July 2007). This study has the approval of institute's ethical committee on animal experimentation.

Plant material

The plant material used is made of *Coccoloba uvifera* (Polygonaceae) leaves harvested in the district of Adéwui (Lome city, Togo) and roots of *Waltheria indica* (Sterculiaceae) harvested in Zanguéra (Maritime region). A voucher specimen, of each plant (*C. uvifera*: TG 07102; *W. indica*: TG 08683) was identified and kept in the herbarium of Laboratory of Botany and Plant Ecology (Faculty of Science/University of Lomé). The samples of *C. uvifera* and *W. indica* were washed, cut into small pieces and dried in conditioner air for two weeks.

Extraction

Hydro alcoholic extract was obtained by soaking plant material (200 g) in ethanol/water (50:50) for 72 hours. The extracts were filtered on Whatman paper filter and evaporated to dryness at 45 °C using a rotary evaporator under vacuum (Büchi Rotavapor R210, Germany). Yield was 19.07 and 11.14% respectively for the leaves of *C. uvifera* and roots of *W. indica*. The extracts were stored in a refrigerator at 4 °C in a sealed vial until use.

Phytochemical screening of the extracts

Qualitative determination of the tannins, flavonoids, alkaloids and saponins was performed on the extracts by chemical analysis according to the method of Harbonne [14].

Total phenols, tannins and flavonoids content

Total phenols were measured in the extracts by the Folin-Ciocalteu method and for the determination of tannins; a second dosage of the phenols was performed after fixing tannins by PVP (Polyvinyl pyrrolidone). Total tannins content was determined by absorbance difference between the first and second assay according to the method of Maksimovic *et al.*, 2005 [15].

Total flavonoids content in the plant extracts was determined by adding 2 ml of aluminum chloride (20 mg/ml) and 6 ml of sodium acetate (50 mg/ml) to 2 ml of extract (1 mg/ml) or rutin. Absorbance was recorded at 440 nm against a blank (2 ml of ethanol instead of the sample) after 150 minutes of incubation. The amount of flavonoids was calculated as a rutin equivalent from the calibration curve of rutin standard solutions, and expressed as mg rutin/100 g of plant material [16].

***In vitro* antioxidant activities of *C. uvifera* and *W. indica* extracts**

AAPH (2, 2'-Azobis 2 Amidino propane Dihydrochloride) test

AAPH is a free radical generator inducing hemolysis in red blood count. The free radical scavenging activity of the extracts was determined by the method described by Bakoma *et al.*, (2012) [17].

Reducing power assay

The assay of the reducing power of extracts was carried out according to the method of Oyaizu (1986)[18]. Briefly, 0.5 ml of the extracts was mixed with 2.5 ml of phosphate buffer (0.2 M, pH 6.6) and 2.5 ml of 1% potassium ferricyanide. The mixture was incubated at 50 °C for 30 min after which 2.5 ml of 10% trichloroacetic acid was added. The resultant solution was centrifuged at 503 g for 10 min. The supernatant(s) were collected and mixed with 2.5 ml of distilled water and 0.5 ml of 0.1% ferric chloride after which the absorbance was read at 700 nm. Ascorbic acid solution was used as the reference antioxidant. An increase in the absorbance of the mixture indicates an enhanced reducing power.

The intensity of the reducing power (in percentage) was calculated from the formula:

$[A_1–A_0/A_0] \times 100$, where A_0 is the absorbance of the control and A_1 is the absorbance of the extract or standard.

Nitric oxide radical scavenging assay

The procedure is based on the method, where sodium nitroprusside in aqueous solution at physiological pH spontaneously generates nitric oxide, which interacts with oxygen to produce nitrite ions that can be estimated using Greiss reagent. Scavengers of nitric oxide compete with oxygen leading to reduced production of nitrite ions. For the experiment, sodium nitroprusside (0.3 ml, 5 mm) in phosphate buffered saline was mixed with 1 ml of different concentrations of hydro alcoholic extract of *C. uvifera* and *W. indica* and ascorbic acid (0.05, 0.1, 0.2, 0.4, 0.8 g/ml) dissolved in methanol and incubated at room temperature for 150 min. The same reaction mixture without the extract but the equivalent amount of methanol served as the control. After the incubation period, 0.5 ml of Griess reagent (1% sulfanilamide, 2% H3PO4 and 0.1% N-(1-naphthyl) ethylenediamine dihydrochloride) was added. The absorbance of the chromophore formed was read at 546 nm [19]. The percentage inhibition activity was calculated from [(A0–A1)/A0] x 100, where A0 is the absorbance of the control and A1 is the absorbance of the extract or standard.

Effect of hydro alcoholic extracts of *C. uvifera* leaves and *W. indica* roots on oral glucose tolerance test

Hyperglycemia was induced in fasted rats (16 hours) by gavage of glucose (2g/kg body weight) at a rate of 5 ml/kg. The animals were divided into 6 groups of 5 rats each. Distilled water (control group), hydro alcoholic extracts of the leaves of *C. uvifera* and the roots of *W. indica* (250 and 500 mg/kg) and metformin (100 mg/kg) were administered by gavage 30 minutes before glucose overload. Blood glucose was measured at different times (0 min: before glucoce loading, 30 min, 60 min, 120 min and 180 min after glucose loading) from the tail vein of rats with the glucometer Gluco D (Germany).

Effect of hydro alcoholic extracts of *C. uvifera* leaves and *W. indica* roots on normoglycemic rats

Four groups of five rats were used; group I served as a control, group II received leaves extract of *C. uvifera*, group III received root extract of *W. indica* at doses of 500 mg/kg and group IV received metformin at a dose of 100 mg/kg. Blood glucose levels were measured at the tail vein before (0 min) and 30, 60, 90, 120, 180 minutes after the administration of the extracts.

Statistical analysis

All data were expressed as means±SEM. Statistical differences between treated groups and controls were determined by analysis of variance (ANOVA) followed by Bonforni test using Graph Pad Prism 5. Differences between groups were considered significant for $P<0.05$.

RESULTS

Phytochemical screening

The results were listed in table 1. Alkaloids, polyphenols (flavonoids and tannins) and especially saponins are the most abundant groups.

Table 1: Phytochemical investigation of *C. uvifera* and *W. indica* hydro alcoholic extracts

Reagent	*C. uvifera*	*W. indica*
Alkaloid identification		
Dragendorf	+	+
Mayer	+	+
Bouchardat	+	+
Flavonoids Identification		
NaOH1/10	-	+
Ferric Chloride	+	+
Tannins identification		
Ferric Chloride 1%	+	+
Lead Acetate	+	+
Copper Sulfate	-	-
saponins identification		
Agitation	++	+++

-: No; +: Trace; ++: Abundant; +++Very abundant

Table 2: Total phenols, tannins and flavonoids content in leaves of C. Uvifera and roots of W. indica

Phytoconstituents	*W. indica*	*C. uvifera*
Total Phenols (mg GA/g)	262.46±17	247.78±9.6
Tannins (mg GA/g)	250.98±22	231.80±14
Flavonoids (mg R/g)	41±3.7	58±4.1

The total phenols and tannins are expressed in mg Gallic acid equivalent/g of extract and flavonoids are expressed in mg equivalent of Rutin/g of extract. The results represent the mean±SEM (n=3).

Total phenols, tannins and flavonoids content in extracts

Phytochemical assays show a high amount of total phenols in the roots of W. indica more than in leaves of C. uvifera (table 2). Tannins are respectively in the extract of W. indica and C. uvifera, 95.66 and 93.60 % of total phenols. Flavonoids content in extract of C. uvifera leaves were higher than that of the extract of W. indica roots 1.41 fold.

Antioxidant activities of extracts

Reducing power of extracts of *C. uvifera* and *W. indica*

The reducing power of the two extracts increased with the concentration. It is found that *W. indica* showed a high reducing power than *C. uvifera*. However, ascorbic acid has a high reducing power than extracts of *W. indica* and *C. uvifera*. (fig. 1)

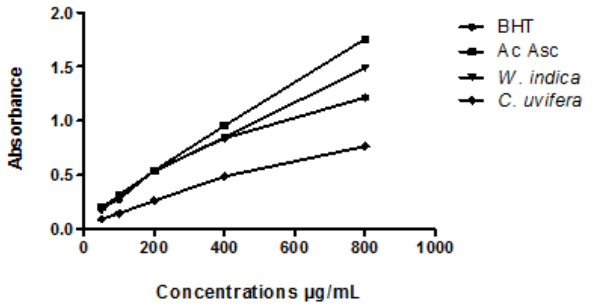


Fig. 1: Reducing power of *Coccoloba uvifera* and *Waltheria indica* extracts compared to those of ascorbic acid (Ac Asc) and Butylated Hydroxy Toluene (BHT) Results are given in mean (n=3)

Activities of the extracts on the red blood cell lysis induced by AAPH *in vitro*

The extracts inhibit the hemolysis of erythrocytes induced by AAPH dose-dependent manner (fig. 2). This inhibitory activity of the extracts (IC_{50}) was compared to that of ascorbic acid (table 3). The antioxidant activity of *W. indica*>Ac Asc>*C. uvifera.*

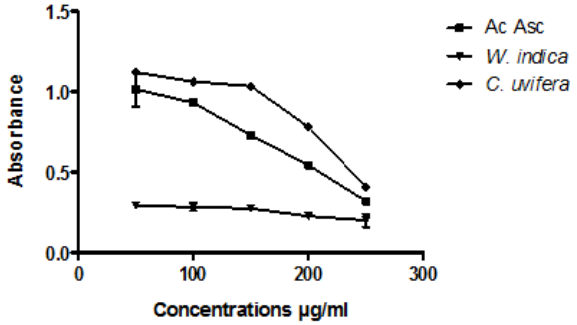


Fig. 2: Curve of the absorbance of the extracts compared to that of ascorbic acid. The results represent the mean±SEM (n=4)

Activity of the extracts of *C. uvifera* and *W. indica* on nitric oxide (NO)

The extracts inhibit the release of nitric oxide dose-dependent manner (fig. 3). This activity is compared to that of ascorbic acid in table 4.

Table 3: *C. uvifera and W. indica* extracts concentration which inhibit 50% of hemolysis

	Ascorbic Ac	*C. uvifera*	*W. indica*
Cl_{50} (µg/ml)	185.5±4.6	240.5±7.4	07.5±0.6

The results represent the mean±SEM (n=4)

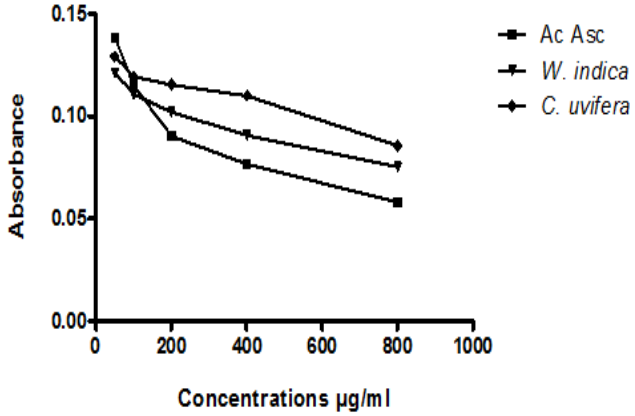


Fig. 3: Activity of the extracts of *C. uvifera* and *W. indica* on nitric oxide (NO)
The results represent the mean (n= 4)

Glucose lowering activities

Effect of extracts of *W. indica* on oral glucose tolerance test

Thirty minutes after the administration of glucose, blood glucose increased and reached its maximum value (peak hyperglycemic) both in control and treated groups (fig. 4A). Hydro alcoholic extracts of *W. indica* reduced significantly hyperglycemia compared to the control group at t_{30}, t_{180} for the dose of 250 mg/kg (P<0.01) and at t_{30}, t_{60} for the doses of 500 mg/kg (P<0.01). The area under the curve (AUC) of the glucose tolerance which measured the total amount of available glucose in the blood also indicates a significant (P<0.01) reduction of blood glucose level compared to control group (fig. 4B).

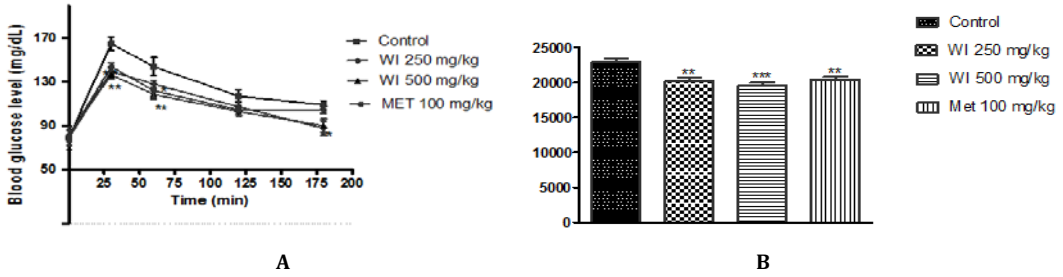
Effect of extracts of *C. uvifera* on oral glucose tolerance test

Hydro alcoholic extracts of the leaves were fairly remarkable 30 min after induction of hyperglycemia compared to the control group (fig. 5A). This inhibitory action was significant at t_{30} for the dose of 250 mg/kg and t_{30} to t_{60} for the dose of 500 mg/kg (P<0.05). The area under the curve (AUC) of the glucose tolerance test indicated no significant reductions in blood glucose compared to the control group (fig. 5B).

Table 4: *Coccoloba uvifera* and *Waltheria indica* extracts concentration which inhibit 50% of nitric oxide release

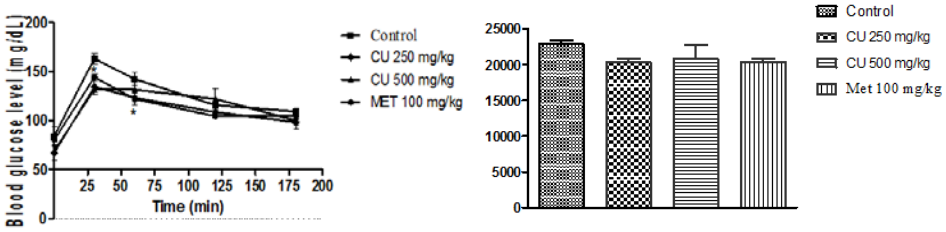
	Ascorbic Ac	*C. uvifera*	*W. indica*
IC_{50} (µg/ml)	381.67±11	791.67±49	548.33±62

IC_{50} = inhibitory concentration 50%



A **B**

Fig. 4: Effect of hydro alcoholic extract of roots of *W. indica* on oral glucose tolerance test (A =blood glucose level *vs* time; B =Area under the blood glucose curve)
At t = 0 min, basal blood glucose was measured in all rats in the treatment groups. 1 hour after oral administration of the extract or the distilled water, hyperglycemia was performed by oral administration of glucose 2 g/kg in rats fasted for 16 hours. T: control who received distilled water; WI 250; WI 500: Treated with *W. indica* extract at doses of 250 and 500 mg/kg. Metformin (MET) was used as reference drug at the dose of 100 mg/kg. The results represent the mean±SEM. ** p<0.01; * p<0.05: compared to the control (T). n = 5



A **B**

Fig. 5: Effect of hydro alcoholic extract of leaves of *C. uvifera* on oral glucose tolerance test (A =blood glucose level vs. time; B =Area under the blood glucose curve)
At t = 0 min, basal blood glucose was measured in all rats in the treatment groups. 1 hour after oral administration of the extract or the distilled water, hyperglycemia was performed by oral administration of glucose 2 g/kg in rats fasted for 16 hours. T: control who received distilled water; CU 250; CU 500: Treated with *C. uvifera* extract at doses of 250 and 500 mg/kg. Metformin (MET) was used as reference drug at the dose of 100 mg/kg. The results represent the mean±SEM. * p<0.05: compared to the control (T) n = 5.

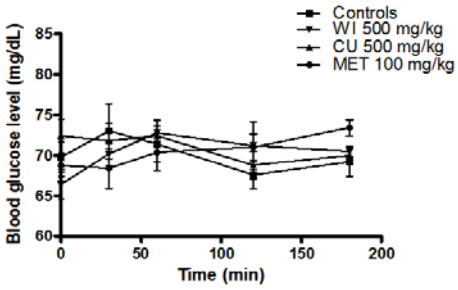


Fig. 6: Effects of hydro alcoholic extracts of *C. uvifera* and *W. indica* on basal blood glucose level
At t_0, basal blood glucose level was measured in all rats. Immediately after sampling, distilled water and extracts of *C. uvifera* and *W. indica* were given to animals fasted for 16 hours. Control received distilled water; WI500 and CU500: Treatment Groups received respectively extract of W. *indica* and *C. uvifera* at a dose of 500 mg/kg; Met: Group treated with metformin 100 mg/kg. The results represent the mean±SEM (n = 5).

Effect of extracts of *C. uvifera* and *W. indica* on basal glucose level

Hydro alcoholic extracts of the leaves of *Coccoloba uvifera* and roots of *Waltheria indica* at a dose of 500 mg/kg have not decreased significantly (P>0.05) basal glucose level in rats, similar effect was observed with metformin (fig. 6)

DISCUSSION

The aim of this work was to evaluate the hypoglycemic activity of leaves extracts *of Coccoloba uvifera* and roots of *Waltheria indica* in fasting normal and glucose loaded hyperglycemic rats and to assess the ability of these plants in antioxidant activities.

In this study, it observed that, oral administration of glucose 2 g/kg in fasted rats caused an increase in blood glucose level (hyperglycemia with a peak at 30 min) which tends to return to normal after 2 hours. This is due to the use of glucose for the needs of the organism or its setting aside under direct action of insulin [20].

After glucose load, *C. uvifera* and *W. indica* extract reduced significantly hyperglycemia. The area under the curve (AUC) of *W. indica* confirm this reduction on blood glucose level suggesting that the extract of *W. indica* induced an increase in peripheral glucose utilization. Metformin used as reference drug prevented the onset of hyperglycemic peak. The mode of action of this reference drug is multifactorial and includes increased glucose utilization by peripheral tissues, insulin action, inhibition of renal and hepatic gluconeogenesis [21]. Moreover, the administration of extracts (at a dose of 500 mg/kg) to fasted rats did not show any significant decrease on basal blood glucose level compared to the control. This suggests that *Coccoloba uvifera* leaves extract and *Waltheria indica*

roots extract have no direct action on insulin secretion. As metformin, extracts of *C. uvifera* and *W. indica* would possess an antihyperglycemic activity. Studies have shown that the activity of plant extracts in the regulation of blood glucose level in animal models of diabetes was correlated to the presence of polyphenols [22]. Phytochemical tests conducted during this study showed the presence of flavonoids, saponins and total phenols in extracts of *C. uvifera* and *W. indica.* The dosage of polyphenol (flavonoids and total phenols) in the extracts shows a high amount of total phenols in the roots of *W. indica* more than in the leaves of *C. uvifera* and the extract of *C. uvifera* is rich in flavonoids (58±*4.1* mgEqR/g) than the extract of *W. indica* (41±3.7 mg EqR/g). This may partly explain the antidiabetic and hypoglycemic activity of the extract.

The diabetic state is characterized by chronic hyperglycemia due to failure of insulin secretion. During the process of oxidation of glucose, there is the generation of free radicals continuously creating damage to the organism causing the oxidation of lipid membranes, tissue damage and DNA breaks. Furthermore, recent studies have shown that hyperglycemia induces overproduction of superoxide by the transport chain and mitochondrial electron. Superoxide overproduction is accompanied by an increased production of nitric oxide (NO) which may also lead to DNA damage. Several reactions succeed and lead to acute endothelial dysfunction in diabetic blood vessels which contributes to the development of cardiovascular disease [23]. It therefore seems clear that hyperglycemia causes the production of oxidative and nitrosative stress (excessive production of NO). So the reducing power of the extracts, the activity on the red blood cell lysis induced by AAPH and the effect on the production of nitric oxide was evaluated. *in vitro*. The hydro alcoholic extracts of the plants inhibit AAPH induced erythrocytes hemolysis dose-dependent manner. The extracts have a reducing power property comparable to that of Butylated Hydroxy Toluene (reference drug). In addition, extracts of *C. uvifera* and *W. indica* inhibit the production of nitric oxide. Therefore, the extracts possess antioxidant and reducing power which could be explained by the presence of phenolic compounds that interact directly on activated oxygen species [24]. Flavonoids and tannins are a class of phenolic compounds with antioxidant power, which may inhibit the formation of free radicals [25, 26]. Chronic hyperglycemia is responsible of the increased production of oxygen free radicals, the contribution of natural antioxidant substances so would help improve the state of oxidative stress in diabetic patients. The high antioxidant activities of roots compared to that of leaves is due to the high rate of total phenols observed in roots than in leaves during the assay.

CONCLUSION

This study has shown the hypoglycemic effect of *Coccoloba uvifera* and *Waltheria indica.* The plants extract contain large amounts of phenols, flavonoids and tannins. The evaluation of the antioxidant activity of the two plants reveals that they have good antioxidant activities. These effects justify the use of *Coccoloba uvifera* and *Waltheria indica* in the treatment of type II diabetes. More detailed pharmacological studies on models of diabetic animals and toxicological studies will be needed for the full use of these plants.

CONFLICT OF INTERESTS

Declared None

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