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## Phytochemical Studies and Evaluation of the Hypoglycemic Activity of the Methanolic Extract of *Tetrapleura tetraptera* Bark ((Schumach & Thonn.) Taub., 1891)

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### Abstract

The aim of this study was to estimate the possible hypoglycemic effect of the methanolic extract of *Tetrapleura tetraptera* bark in rats made diabetic by streptozotocin. Initially, our study focused on a triphytochemistry of our extract. It showed that the methanolic bark extract is richer in polyphenols, flavonoids, quinones, saponosides, stereol-polyterpens, gall tannins and catechic tannins but poor in alkaloids and anthocyanins. We found that the methanolic extract of the bark has a dose-dependent hypoglycemic activity. First, experimental diabetes was induced in male Wistar rats by intraperitoneal injection of STZ (50mg/kg). Treatment with a methanolic extract of the plant was given orally at daily doses of 250, 500, 750 mg/kg/pc for 14 days. The results obtained, clearly show that streptozotocin induces in animals, a diabetes characterized by hyperglycemia. However, the oral administration of doses of methanolic bark extract for 14 days caused a clear decrease in serum glucose concentration.

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### Keywords

Phytochemical studies, Activité Hypoglycémiant, *Tetrapleura tetraptera* bark.

### Introduction

Since 1980, scientists from all over the world have largely focused on the search for new therapeutic molecules of natural origin. This trend can be largely explained by the urgent need for new treatments for several reasons, including the increasing and sometimes severe side effects of synthetic drugs (Schlienger, 2014).

Among the plants that have gained great interest and have been the subject of several research studies are the plants with anti-diabetic activity, which have the power to regulate the blood sugar level of diabetic patients, the

number of which is constantly increasing. According to scientific reviews, these plants mainly belong to the following families; *Leguminosae*, *Lamiaceae*, *Liliaceae*, *Cucurbitaceae*, *Asteraceae*, *Moraceae*, *Rosaceae* and *Araliaceae*. In addition, the most active plants are *Allium sativum*, *Gymnema sylvestre*, *Citrullus colocynthis*, *Trigonella foenum greacum*, *Momordica charantia* and *Ficus bengalensis* (Patel *et al.*, 2012).

This is also the case for *Tetrapleura tetraptera*, a deciduous tree belonging to the family Fabaceae. It is generally distributed in the lowland forests of tropical Africa, particularly in West, Central and East Africa

(Ironi *et al.*, 2013). Much work has been done on the shell, pulp and seed of the fruit of *Tetrapleura tetraptera* (Akin-Idowu *et al.*, 2011; Moukette *et al.*, 2015).

Furthermore, the flowers, fruits, bark are used in ethnomedicine for the treatment of several diseases such as diabetes, hypertension, intestinal parasites, malaria, asthma, epilepsy, schistosomiasis (Ironi *et al.*, 2013). In traditional cosmetics, the fruits and flowers of *Tetrapleura tetraptera* are used to make perfumes and ointments (Orwa, 2009).

The fruits are also used as a mosquito repellent. They are also powerful plant molluscides to prevent transmission of bilharzia (Aladesanmi, 2007). In the area of food, the fruits are used in Central and West Africa to prepare soup for nursing mothers from the first two days of life.

The present work focuses on the evaluation of the anti-diabetic activity of the methanolic extract of *Tetrapleura tetraptera* barks on rats made diabetic by streptozotocin (Ojewole et Adewunmi, 2004; Moukette *et al.*, 2015).

The present work focuses on the evaluation of the anti-diabetic activity of methanolic extract of *Tetrapleura tetraptera* barks on rats made diabetic by streptozotocin.

## Materials and Methods

### Plant material

The barks of *Tetrapleura tetraptera* (Schum. & Thonn.) Taub., 1891, (Fabaceae), harvested in October 2018 in Kassiapleu near Man (western of Côte d'Ivoire) have been identified by the National Center of Floristry at the University Felix Houphouët Boigny (Cocody-Abidjan). A specimen of the plant was deposited in the herbarium of this Center.

### Animal Material

The animal material used consists of rats of the *Rattus norvegicus* (Muridae) strain wistar type (males) with a weight ranging from 95 to 175 g, with no lesions in the eyes.

They come from the vivarium of the Research Unit in Applied Sciences for Production and Animal / Human Health (URSASAH) UFR of the Sciences of Nature of the University Nangui Abrogoua (Ivory Coast).

### Preparation of aqueous extract

100 g powder of bark of *T. tetraptera* were macerated for 24 hours in 1L of distilled water (Olakunle *et al.*, 2005). The macerate has been wrung into a square of sterile tissue, filtered successively on cotton wool and one fold on filter paper (Whatman paper® 2mm). The filtrate was dried slowly in the stove at 50°C. The powder obtained was stored in a hermetically sealed jar and refrigerated at 4°C (Zirihi *et al.*, 2003).

### Preparation of methanol extract

It was carried out using modified Olakunle *et al.*, (2005) method. A mass of 20g of plant powder was added in 100ml of methanol and subjected to maceration for 72 hours. The macerate was treated according to the same procedure like the aqueous extract.

### Phytochemical screening

#### Test for alkaloids (reactions Dragendorff and Bouchardat)

Six milliliters of plant extract were evaporated. The residue was taken up in six milliliters of alcohol at 60 ° and the alcoholic solution thus obtained was divided into two test tubes. In the first tube was added two drops of Dragendorff reagent. The appearance of a precipitate or an orange color indicated the presence of alkaloids.

In the second tube was added two drops of reagent Bouchardat. The appearance of a reddish brown color indicated a positive reaction to the presence of alkaloids (Kpemissi, 2007).

#### Test for polyphenols

Two milliliter of extract was added a drop of alcoholic solution of ferric chloride at 2%. The appearance of a dark green or lighter or darker blue color indicated the presence of polyphenolic derivatives (Bidie *et al.*, 2011).

#### Test for flavonoids

For this research, two milliliter of the extract was evaporated to dryness in a porcelain dish on a sand bath. The residue was taken after cooling in five milliliters hydrochloric alcohol half. The successive addition of three magnesium shavings and three drops of isoamyl alcohol showed an intense pink or violet in the presence of flavonoids (Munmi *et al.*, 2013).

### **Test for saponosides**

A volume of two milliliters of extract was evaporated and taken up in five milliliters of water. After vigorous stirring, the foaming of more than one centimeter, stable and persistent high for 30 minutes indicated the presence of saponins (Munmi *et al.*, 2013).

### **Test for catechol or condensed tannins (reaction Stiasny)**

A volume of five milliliter of extract was evaporated and an amount of 10 ml of a reagent solution Stiasny was added to the residue.

This mixture was placed in a water bath at 80 ° C for 30 minutes and was cooled to room temperature. Positive feedback had resulted in the formation of large flakes brown clear or dirty precipitates (Yéo *et al.*, 2014).

### **Search for catechic or condensed tannins**

Catechic tannins were identified by Stiasny's reagent (Formol 30%, concentrated HCl: (1/0.5; v/v). 5 mL of extract was evaporated to dryness in a capsule on a sand bath without charring.

After adding 15 mL of Stiasny's reagent to the residue, the mixture was kept in a water bath at 80 °C for 30 min. The observation of a coarse flake precipitate characterizes the catechic tannins (Yéo *et al.*, 2014).

### **Test for Gallic tannins**

The above solution was saturated and one or two drops of alcoholic solution of iron chloride to 2 % have been added. The positive response has led to the appearance of blue-black coloration characteristic intense tannins Gallic (Yéo *et al.*, 2014).

### **Test for sterols and polystyrenes (reaction LIEBERMANN)**

After evaporation to dryness 5mL of solution in a capsule on a sand bath without charring, the residue was dissolved in hot acetic anhydride and 1 mL in a test tube, we poured cautiously with 0.5 mL of concentrated sulfuric acid along the tube wall to the solution. The applications to the interphase of a purple or purple ring, turning blue to green, indicate a positive reaction (Yéo *et al.*, 2014).

### **Quinonic substances research**

The Borntraeger reagent makes it possible to identify free quinone substances. The combined quinonics are identified by hydrolysis. To do this, 2 mL of each extract solution and fraction are first evaporated to dryness in a sand bath capsule without charring, then the residue is triturated in 5 mL of hydrochloric acid at 1/5. Then the solution obtained is brought to the boiling water bath for half an hour.

Finally, after cooling on a current of cold water, the hydrolyzate is extracted with 20 ml of chloroform and the chloroform phase is collected in another test tube supplemented with 0.5 ml of ammonia diluted by half. The appearance of a color ranging from red to purple indicates the presence of quinones (Yéo *et al.*, 2014).

### **Test for Gallic tannins**

The above solution was saturated and one or two drops of alcoholic solution of iron chloride to 2 % have been added. The positive response has led to the appearance of blue-black coloration characteristic intense tannins Gallic (Yéo *et al.*, 2014).

### **Antidiabetic activity**

#### **Weighing and packaging**

The animals were weighed using a precision scale at fixed times before the induction of diabetes (beginning of the manipulation) and every day after the induction of diabetes.

These animals were kept in a room in plastic cages with a stainless steel lid. A thick layer of sawdust is deposited at the bottom of the cages, renewed every 2 to 3 days, at a constant temperature of 27 ° C, a photoperiod of 12 hours of natural light and 12 hours of darkness.

The hygrometry was 50 to 55% and the animals had free access to water and food (granules, 15% protein, and 4 % fat) provided by Faci-Abidjan.

#### **Glucose assay**

The evolution of the glycemia of the rats of the different groups is controlled from the first day of the treatment, and until the end of the treatment. According to a program identical to that of weighing.

## Induction of diabetes

The animals were deprived of food for 16 hours but had water at will. Diabetes was induced by injection of streptozotocin (STZ, Sigma S0130-500MG) according to the method described by. They received intraperitoneally a single dose of 50 mg / kg of body weight, ie a volume of 2 ml / kg of the STZ solution freshly prepared in 0.1M sodium citrate buffer pH 4.5.

After induction, the rats received a 5% anhydrous glucose solution overnight to overcome hypoglycemia induced by STZ action. Seventy-two hours (72 h) after the injection, a blood sample was taken by caudal amputation to measure the blood glucose. Rats with blood glucose levels greater than 2 g / L were retained for the rest of the experiment.

## Animal treatment

After induction of diabetes, all diabetic rats were divided into 6 lots of 3 rats each and kept under the same conditions. The start of treatment with methanolic extracts of *Tetrapleura tetraptera* bark of the plant or with distilled water for controls begins 3 days after the induction of diabetes and lasts 14 days (duration of treatment).

## Animal groups

Lot 1 (Normal Control): consists of control rats having received only distilled water throughout the experiment.

Lot 2 (Diabetic control): consists of diabetic rats, having received only distilled water throughout the experiment.

Lot 3: (Diabetic + Glibenclamide): consists of diabetic rats treated with Glibenclamide the reference substance at a dose of 10 mg / kg bw.

Lot 4 (Diabetic + EMec): consists of diabetic rats treated with the methanolic bark extract (EMfe) at doses of 250 mg / kg bw respectively.

Lot 5 (Diabetic + EMec): consists of diabetic rats treated with the methanolic extract of the Bark at the respective doses of 500 mg / kg bw.

Lot 6 (Diabetic + EMec): consists of diabetic rats treated with the methanolic extract of the bark at the respective doses of 750 mg / kg bw.

## Blood sampling in rats

The sample is taken from the retro-orbital sinus of the eye (richly vascularized cartilaginous region) using a glass Pasteur pipette immersed in an alcoholic solution. These samples are taken from fasted and anesthetized rats. The collected blood was respectively placed in numbered heparinized tubes and then centrifuged at 3000 rpm for 5 minutes to obtain the serum allowing the determination of blood glucose and other serum parameters such as total cholesterol, triglycerides HDL. Serum glucose analysis of all groups of rats was performed by the Glucose Oxidase Peroxidase (GOD-POD) method using a glucose estimation kit.

Other serum estimation performed by spectrophotometry using a standard kit and following the instructions provided. Serum triglycerides, total cholesterol, HDL cholesterol (high density lipoprotein) were analyzed using standard kits.

## Body weight changes

In order to determine the influence of our treatments on the body weight and growth of the rats, we monitored the body weight of the control and experimental rats on a daily basis for 14 days. The body weight was measured with a scale in grams (g) and the changes in body weight of the rats compared to day 1 were expressed as a percentage (%) and calculated according to the following formula change in body weight (%) =  $(PJ - PJ_0 / PJ_0) * 100$

## Results and Discussion

### Phytochemical screening of the methanolic extract of *Tetrapleura tetraptera* bark

The screening of the chemical constituents of the methanolic extract of *Tetrapleura tetraptera* bark revealed the presence of polyphenols, tannins, flavonoids, saponins, quinones, sterols and polyterpenes, including the absence of alkaloids with the Dragendorff reagent and anthocyanins (Table I).

### Antidiabetic activity of the methanolic extract of the barks of *Tetrapleura tetraptera*

#### Weight variations in the presence of EMec

J0 corresponds to the day when the initial weight of the rats was known (control =  $113.67 \pm 5.11$ ), (diabetics

125.00±6.00), (STZ+Gliben =117.33±9.11), (EMec250 mg/kg/PC =125.67±4.44), (EMec500mg/kg/PC=125.00±11.33), (EMec750mg/kg/PC=155.67±12.89).

On day 3, the weight of the rats in the control group showed a gain of 2.05%, the untreated diabetic group showed a reduction of -5.34%, the group treated with STZ+Gliben showed a decrease in weight of -12.21%, as was observed in the groups treated with the extract at doses of 250, 500 and 750 mg/kg bw with reduction rates of -8.22%, -6.4% and -11.78%.

At day 7, the control group showed a weight gain of 9.67%, compared to a reduction of -3.74%. In the group treated with STZ+Gliben, there was a decrease of -14.48% and in rats in the groups treated with the extract at doses of 250, 500 and 750 mg/kg bw, the weights decreased by -11.67%, -18.13% and -27.84%.

At d14, the weight of the rats in the control group increased by 16.12%, and in the untreated diabetic group a reduction of -2.94% was observed. The treated groups of rats showed a decrease of -10.08%, -18.40% and -25.91% for the doses of 250, 500 and 750 mg/kg bw respectively of -11.67%, -18.13% and -27.84%.

#### **Evaluation of the blood sugar level of the methanolic extract of *Tetrapleura tetraptera* bark**

Compared to the control group, there is a significant difference between the different groups of rats studied. This difference is 3.83±0.65 with the diabetic group, 3.20±0.65 with the STZ +Gliben group, 2.74±0.65 with the group treated with MEc 250 mg/kg /pc, 3.33±0.65 with the MEc 500 mg/kg /pc group and 3.43±0.65 compared to the group treated with MEc 750 mg/kg /pc at d3. (P < 0.05). There was a significant difference between the mean of the diabetic group and the other groups tested. The values obtained are control 3.56±0.40; -2.35±0.40; -2.96±0.40; 2.97±0.40 and -3.08±0.40 respectively compared to STZ+Gliben, MEc 250 mg/kg/pc treated, MEc 500 mg/kg/pc treated and MEc 750 mg/kg/pc treated groups. (P < 0.05).

There was a significant difference : between the mean of the diabetic group and the control group 3.17±0.12; between that of the STZ+gliben group and the control group 0.73±0.12; between that of the STZ+gliben group and the diabetic group -2.43±0.12; between that of the group treated with MEc 250 mg/kg/bw and the diabetic group -3.08±0.12; between that of the group treated with MEc 250 mg/kg/bw and the STZ+gliben group -

0.64±0.12; between the MEc 500 mg/kg /bw group and the diabetic group -3.22±0.12; between the MEc 500 mg/kg /bw group and the STZ+gliben group -0.78±0.12; between the MEc 750 mg/kg /bw group and the diabetic group -3.23±0.12; and between the MEc 750 mg/kg /bw group and the STZ+gliben group -0.79±0.12 at d14. (P < 0, 05)

#### **Lipid profile**

Serum concentrations of total cholesterol, HDL-cholesterol and triglycerides are shown in Figures 3, 4 and 5 respectively.

#### **Evolution of serum Total Cholesterol concentration in EMec treated rats**

At d3, there was an increase in serum Total Cholesterol concentration in the control group (0.88±0.12 to 0.95±0.04) and in the untreated diabetic rats (0.95±0.25 to 0.99±0.13), a decrease in serum Total Cholesterol concentration in the rats treated with STZ+gliben from (1, 03±0.08 to 0.95±0.14), and in those rats treated with EMec extract at different doses of 250 mg/kg / Pc (0.81±0.36 to 0.82±0.19), 500 (1.03±0.06 to 1.01±0.08) mg/kg / Pc and 750 mg/kg / Pc (0.88±0.21 to 0.77±0.02) compared to d0. At P < 0.05, there was no significant difference between group means.

At d7, there was a decrease in serum Total Cholesterol concentration in all groups: in the control group (0.56±0.05), in the group not treated with (0.93±0.07), in the rats treated with STZ+gliben (0.55±0.19) and in the groups treated with EMec extracts at the different doses of 250, 500 and 750 mg/kg / Pc with values of 0.58±0.06; 0.73±0.30 and 0.67±0.29 respectively. At P < 0.05, there was no significant difference between the group mean. At d14, there was an increase in total cholesterol in all groups: the control (0.85±0.03), the untreated diabetic with a value of 1.14±0.14, in the STZ+gliben group (0.90±0.01), the groups treated with the different doses of 250 mg/kg / Pc (0.77±0.11), 500 mg/kg / Pc (0.78±0.03) and 750 mg/kg / Pc of EMec (1.02±0.16) at d14. At P < 0.05, there was no significant difference between the mean of the groups (P < 0.05).

#### **Evolution of serum HDL cholesterol concentration in EMec treated rats**

On day 3, there was a decrease in HDL cholesterol levels in the glibenclamide-treated groups (0.80±0.13 to 0.66±0.20) and in the EMec-treated groups at 500 mg/kg

bw ( $0.69\pm 0.19$  to  $0.53\pm 0.26$ ) and 750 mg/kg bw ( $0.51\pm 0.26$  to  $0.45\pm 0.06$ ) while there was a decrease in HDL cholesterol levels in the EMec-treated groups at  $0.45\pm 0.06$ , 06) while HDL cholesterol levels were increased in the control group ( $0.49\pm 0.20$  to  $0.65\pm 0.26$ ), in the group treated with 250 mg/kg bw EMec ( $0.30\pm 0.13$  to  $0.60\pm 0.19$ ) and in the untreated diabetic group ( $0.53\pm 0.32$  to  $0.90\pm 0.19$ ). At  $P < 0.05$ , there was no significant difference between the mean of the groups.

At d7, there was a decrease in HDL cholesterol in the control group ( $0.29\pm 0.02$ ), in the untreated diabetic group ( $0.60\pm 0.15$ ), in the STZ+ gliben treated groups ( $0.34\pm 0.12$ ), and in the group treated with 250 mg/kg bw EMec ( $0.41\pm 0.14$ ), while there was an increase in HDL cholesterol in the 500 and 750 mg/kg bw extract groups with values of  $0.64\pm 0.32$  and  $0.46\pm 0.24$ , respectively, compared to d3. At  $P < 0.05$ , the difference was not significant between the group means.

At d14, an increase in HDL cholesterol was observed in the control group ( $0.37\pm 0.04$ ) and a decrease in HDL in all other groups: untreated diabetic ( $0.58\pm 0.04$ ), STZ+ gliben-treated ( $0.33\pm 0.01$ ) and EMec-treated at doses of 250 ( $0.29\pm 0.02$ ), 500 ( $0.36\pm 0.01$ ) and 750 mg/kg / Pc ( $0.38\pm 0.12$ ) compared with d7. At  $P < 0.05$ , there was no significant difference between the group means.

### Evolution of serum triglyceride concentration in EMec treated rats

In the presence of EMec, on d3, there was a decrease in Triglyceride levels in the control group ( $1.07\pm 0.13$  to  $0.87\pm 0.15$ ) an increase in Triglyceride levels was noted in all groups: Untreated diabetic group ( $0.93\pm 0.08$  to  $1.61\pm 0.44$ ), STZ+gliben group ( $1.13\pm 0.10$  to  $1.67\pm 0.53$ ), and diabetic group treated with doses 250 ( $1.02\pm 0.16$  to  $1.22\pm 0.40$ ), 500 ( $1.04\pm 0.00$  to  $1.17\pm 0.34$ ) and 750 mg/kg bw, ( $1.01\pm 0.10$  to  $1.66\pm 0.18$ ) compared to the groups at d0. There was no significant difference between group means at  $P < 0.05$ . At d7, there is a decrease in Triglyceride levels in all groups: in the control group ( $0.65\pm 0.04$ ), in the untreated diabetic group ( $0.81\pm 0.04$ ), the STZ+gliben group ( $0.67\pm 0.06$ ), the diabetic groups treated with EMec at the doses of 250, 500 and 750 mg/kg bw, with respective values of  $0.69\pm 0.04$ ;  $0.81\pm 0.18$  and  $0.80\pm 0.17$  compared to the groups at d3. The difference was not significant between group means at  $P < 0.05$ . At d14, there was still a decrease in

Triglyceride levels in all groups: untreated diabetics ( $0.70\pm 0.15$ ) and diabetics treated with EMec at doses of 250 ( $0.59\pm 0.10$ ), 500 ( $0.66\pm 0.12$ ) and 750 mg/kg bw ( $0.55\pm 0.05$ ) compared to d7. An increase in Triglycerides was noted in the control group ( $0.88\pm 0.02$ ) and in the STZ+gliben group ( $0.69\pm 0.03$ ) compared to d7. There was no significant difference between group means at  $P < 0.05$ .

In this work, maceration was carried out on the powdered bark of *Tetrapleura tetraptera*. Methanol was used as solvent. The yield value was calculated in relation to the initial mass of *Tetrapleura tetraptera* powder. The methanolic extract of the barks has a yield of 12.85%. According to Siddhuraju et Becker (2003), extraction of *T. tetraptera* barks with methanol reveals to be a good solvent for its ability to extract chemical compounds with antioxidant and hypoglycaemic properties such as flavonoids, tannins, saponins, triterpenoids. For example, gallic and catechic tannins are used for their antioxidant Yessoufou *et al.*, (2013) and haemostatic activities (Agunu, 2005; Bruneton, 2009).

For treated diabetic rats (Figure 1), we observed a decrease in body weight after STZ injection ( $121.33\pm 27.78$  versus  $125.00\pm 6.00$ ). Our results are in agreement with those reported by Pari et Latha, (2005), who found that in male Wistar albino rats, STZ injection caused a significant decrease in body weight ( $137\pm 7$  versus  $181\pm 7$ ). This decrease is due to muscle destruction or degeneration of structural proteins (Salahuddin et Jalalpure, 2009).

Similarly, Arunachalam et Parimelazhagan (2013), showed that upon injection of streptozotocin to normal rats, there was an increase in blood glucose and decrease in insulin level due to streptozotocin-induced abnormalities in  $\beta$ -cell function. However, after administration of methanolic extracts of *T. tetraptera* bark at doses of 250, 500 and 750 mg/kg, there was a significant decrease in blood glucose levels.

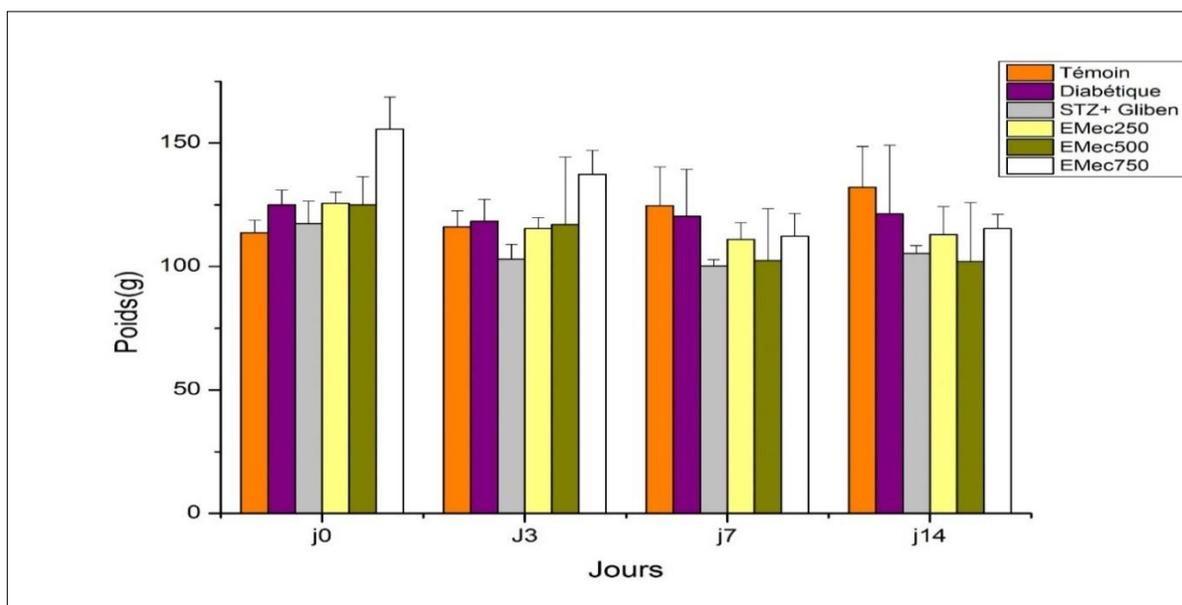
This indicates that the methanolic extracts of bark decreased the blood sugar level of the groups of animals at 250, 500 and 750 mg/kg respectively by ( $3.05\pm 0.59$  to  $0.51\pm 0.11$ ); ( $3.63\pm 0.31$  to  $0.36\pm 0.15$ ); ( $3.74\pm 1.13$  to  $0.35\pm 0.11$ ).

**Table.1** Phytochemical analysis of the methanolic extract of *Tetrapleura tetraptera* bark

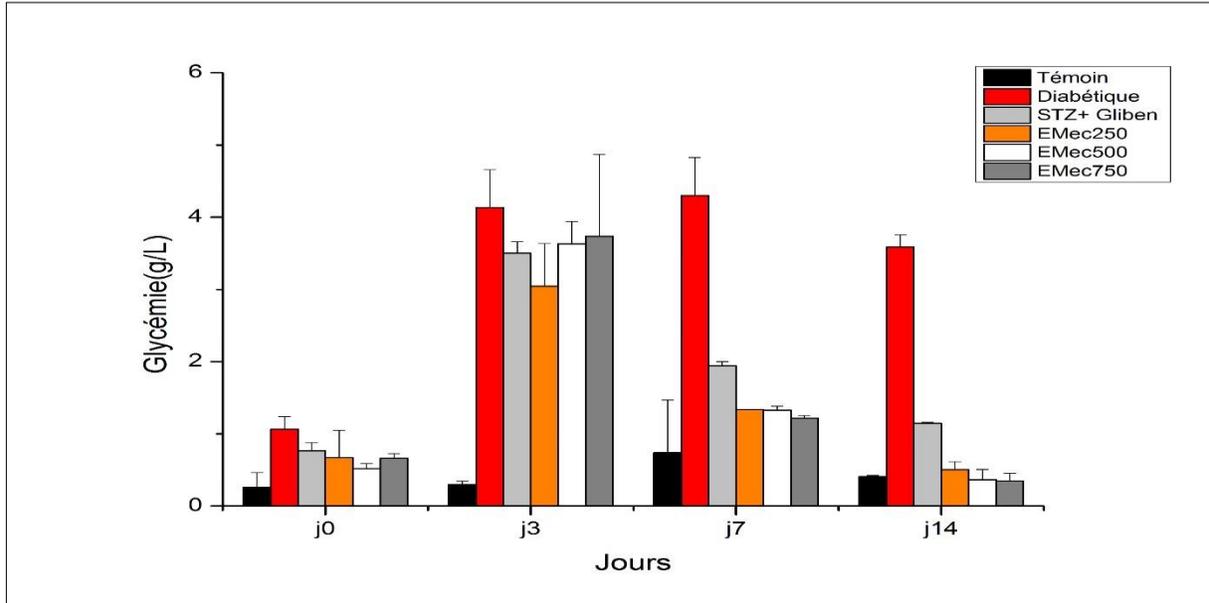
Extraits		EMec
Alcaloïdes	Bouchardâf	+
	Dragendorff	-
Anthocyanes		-
Flavonoïdes		+
Polyphénols		+
Quinones		+
Saponosides		+
Tannins galliques		+
Tannins catéchiques		+
Stérols et Polyterpènes		+

Absence: (-) Presence: (+) EMec: Methanolic extract of bark

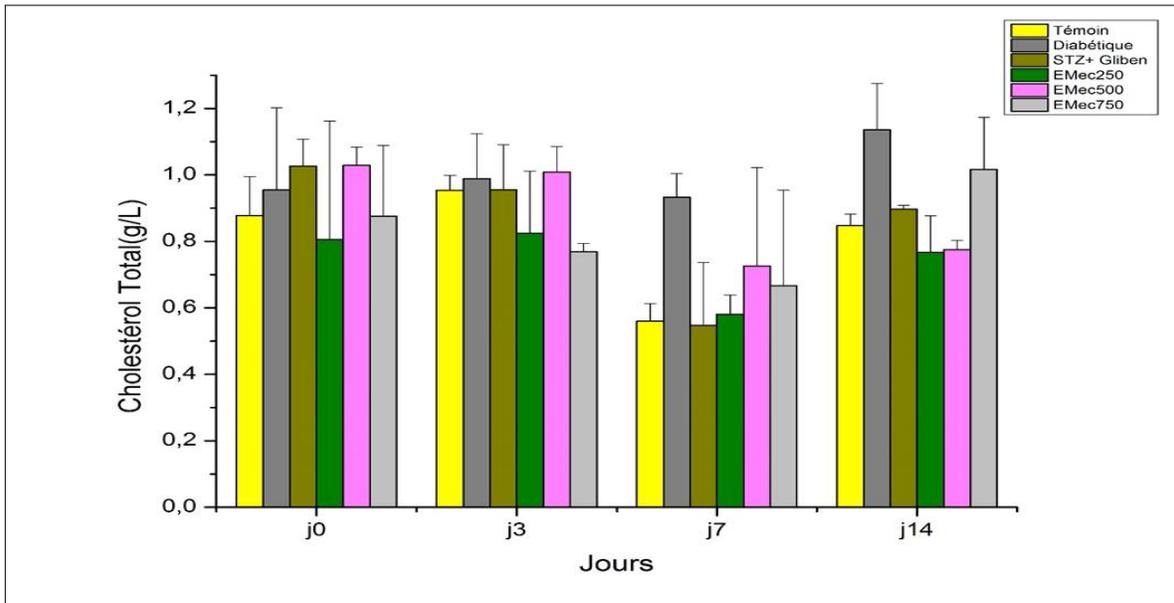
**Fig.1** Changes in body weight (g) in rats treated with *Tetrapleura tetraptera* MEc as a function of time (Days). Values are expressed as mean± SEM (n=3). P < 0.05



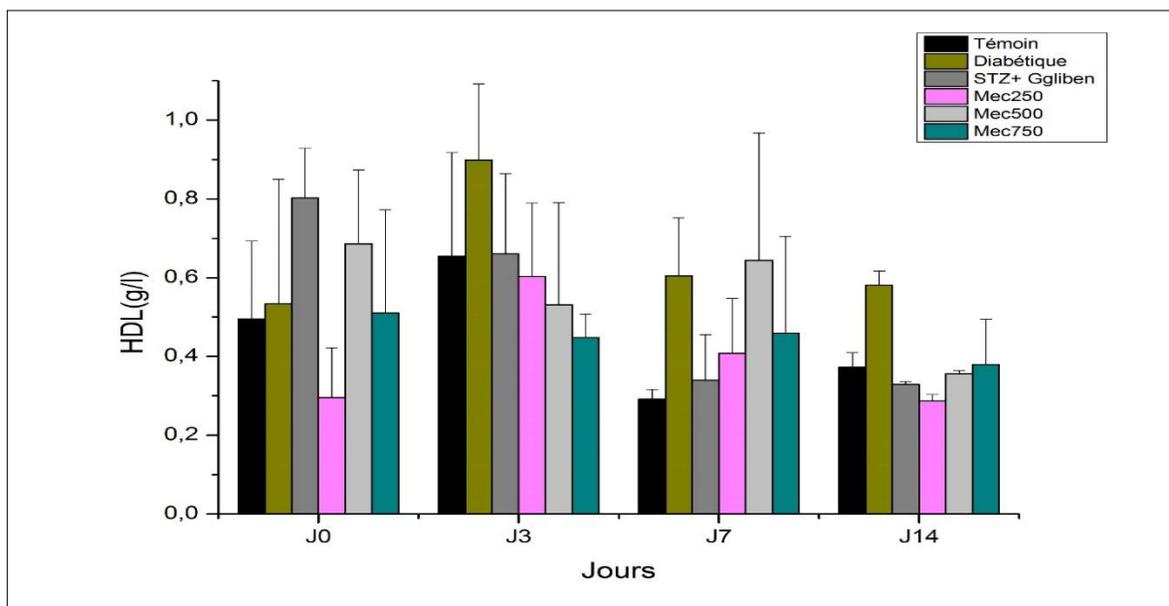
**Fig.2** Change in blood glucose (g/L) in rats treated with *Tetrapleura tetraptera* MEc as a function of time (Days). Results are expressed as mean±SEM (n=3). p < 0.05



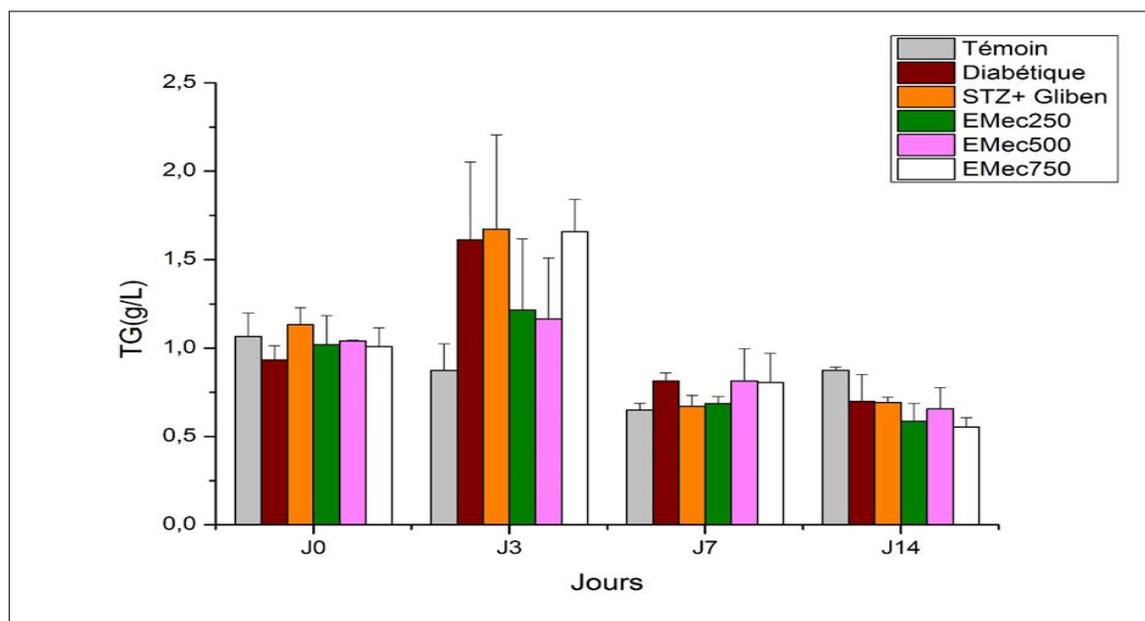
**Fig.3** Changes in serum Total Cholesterol concentration (g/L) in rats treated with *T. tetraptera* MEc as a function of time (Days). Results are expressed as mean ± SEM (n=3). p < 0.05



**Fig.4** Changes in serum HDL cholesterol concentrations (g/L) in rats treated with *T. tetraptera* MEc as a function of time (days). Results are expressed as mean  $\pm$  SEM (n=3).  $p < 0.05$



**Fig.5** Changes in serum triglyceride concentration in rats treated with *Tetrapleura tetraptera* MEc as a function of time (days).



This observation suggests that the substances responsible for the hypoglycaemic activity of *T. tetraptera* are probably of polar nature and more soluble in the organic solvent (methanol). The methanolic extract of a plant usually contains many chemical compounds capable of producing specific biological activities (Ojewole, 2003). It is known that in diabetes mellitus, the sites and

pharmacological mechanisms of drug interventions in biochemical processes are diverse (Marles, 1995). It is likely that this potential diversity in the hypoglycaemic mechanism of drug action also applies to methanolic extracts of *T. tetraptera* bark. The bioactive molecules in these extracts probably have an insulin-like effect, which would be to stimulate pancreatic  $\beta$ -cells to produce

insulin, thereby lowering blood glucose levels. Similar observations have been reported by several authors (Fuentes *et al.*, 2004; Sepici *et al.*, 2004). Like the plant extracts, Glibenclamide also produces a significant reduction in blood glucose levels in normal fasting rats.

The present results appear to be in agreement with the earlier suggestion by (Jackson et Bresser (1981), that sulphonilureas such as glibenclamide have extra-pancreatic effects.

The results of this work indicate that methanolic extracts of *Tetrapleura tetraptera* bark have hypoglycaemic activity. This activity could be attributed to certain compounds of different natures present in the plant. On the basis of these results, it can be assumed that *Tetrapleura tetraptera* decreases the level of glucose in the blood and increases the level of insulin secreted by the pancreas.

The hypotriglyceridaemia noted in the diabetic group treated with methanolic extract compared to the untreated group is concomitant with the reduction in their synthesis in the liver. Similar results have been reported by other authors, in rats made diabetic by streptozotocin (Mukherjee et Patil, 2012). Similarly, our results are in agreement with those obtained by Ben *et al.*, (2007).

The reduction in plasma triglyceride observed in diabetic rats treated with methanolic extract of *T. tetraptera* could be explained by the decrease in fatty acid synthesis.

These results are similar to those of Arie *et al.*, (1997), obtained with both doses (200 mg/kg/day and 400 mg/kg/day) of the methanolic extract of the leaves of *R. alaternus*. These authors state that the effect of the methanolic extract on the decrease of the plasma cholesterol level may be due to the stimulation of insulin secretion, the latter also acting by activating LCAT (Lecithin Cholesterol Acyl Transferase). This enzyme is responsible for the transfer of free cholesterol to esterified cholesterol which migrates to the centre of the lipoprotein (HDL), thus promoting a decrease in its plasma concentration.

In addition, a very large number of studies have shown the hypolipidemic effect of several flavonoids and other phenolic compounds (Shao *et al.*, 2007; De Souza *et al.*, 2008; Deng *et al.*, 2008). The hypolipidemic effect of *T. tetraptera* is thought to be related to its richness in secondary metabolites and the structural diversity of the

latter, in particular terpenes, phenolic compounds consisting mainly of flavonoids and tannins (Veerapur *et al.*, 1996).

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The methanolic extract of *Tetrapleura tetraptera* bark showed good hypoglycaemic and hypolipidaemic activity in rats made diabetic by streptozotocin by decreasing the serum concentration of glucose, total cholesterol and triglycerides. Our work has highlighted the beneficial effects of the administration of the methanolic extract of the plant due to the richness of phytochemical compounds with the presence of several compounds such as tannins, total phenols and flavonoids selected in the decrease of glycaemia and lipid profile. The present study validates the use of *T. tetraptera* bark in traditional medicine in Côte d'Ivoire and demonstrates that the methanol extract of the bark of the plant contains phytochemicals that show very impressive potency and promising prospects in the management of diabetes and its complications. However, further studies are needed to identify the biologically active molecules to give the precise molecular mechanism(s) responsible for these effects

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