

Original Article

PHYTOCHEMICAL STUDY AND ANTIOXIDANT, ANTIBACTERIAL AND ANTIDIABETIC ACTIVITIES OF *FLACOURTIA INDICA* LEAVES EXTRACTS FROM THE TOGOLESE FLORA

AYEFOUNIN OGOUBI¹, KODJO SELOM EVENAMEDE^{1*}, KAFUI KPEGBA¹, OUDJANIYOBİ SIMALOU¹, AMEGNONA AGBONON²

¹Laboratoire de Chimie Organique et des Substances Naturelles (Lab COSNat), Faculte des Sciences, Universite de Lome, 01 BP-1515 Lome 01, Lome-Togo, ²Laboratoire de Physiologie/Pharmacologie, Faculte des Sciences, Universite de Lome 01 BP-1515 Lome 01, Lome-Togo
*Corresponding author: Kodjo Selom Evenamede; *Email: ev_selom@yahoo.fr

Received: 06 Apr 2023, Revised and Accepted: 13 Jun 2023

ABSTRACT

Objective: Faced with the devastating effect of diabetes, the search for new natural antidiabetic molecules is an exploratory avenue.

Methods: In this study, the leaves of *Flacourtia indica* were collected from the Togolese flora, and their antioxidant activity was evaluated using DPPH and FRAP assays, following a phytochemical screening conducted through colorimetric and precipitation reactions. The content of total phenols was determined using Folin-Ciocalteu's reagent and flavonoids by aluminum trichloride. The antibacterial activity of the extracts was evaluated using the method of dilution in agar wells with Muller-Hinton® agar. Additionally, the extracts' antidiabetic effect was evaluated in rats made diabetic by alloxan at a dose of 120 mg/kg. b.w.

Results: The results of our work showed the presence of flavonoids, tannins, anthocyanins, and saponins in the extracts. The best polyphenol and flavonoid contents were measured in the hydroethanolic extract and were 186.46±0.308 mg EqAG/g and 464.14±17.043 mgEqQ/g. ES respectively. The hydroethanolic extract has a higher antioxidant power with an IC₅₀ = 110.22 µg/ml. The reducing powers of the ferric ions were 3706±12.124 and 3777±9.238 µmol/ml, respectively for the hydroethanolic and aqueous extracts. The extracts were active against *Staphylococcus aureus* and are bacteriostatic. The hydroethanolic extract of the leaves of *Flacourtia indica* significantly reduced (p = 0.0014) blood glucose levels in diabetic rats.

Conclusion: This plant can therefore be a potential medicine in the treatment of diabetes.

Keywords: *Flacourtia indica*, Alloxan, Diabetes, Antioxidant, Polyphenols

© 2023 The Authors. Published by Innovare Academic Sciences Pvt Ltd. This is an open access article under the CC BY license (<https://creativecommons.org/licenses/by/4.0/>)
DOI: <https://dx.doi.org/10.22159/ijpps.2023v15i8.48035>. Journal homepage: <https://innovareacademics.in/journals/index.php/ijpps>.

INTRODUCTION

These days, human beings are facing diseases of all kinds and the management of health issues is proving to be a real societal problem, especially for countries where the economic system is weak [1]. Diabetes is one of the deadly diseases in the world. Diabetes is characterized by hyperglycemia and chronic complications, including retinopathy, kidney damage, and heart damage. It has become the leading cause of death on a global scale. Due to the fact that diabetes is a chronic infection, diabetic patients are bound to a lifelong, consistent and very expensive treatment in a hospital environment [1]. Furthermore, although oral hypoglycemic agents/insulin are the most important elements in the treatment of diabetes and effective in checking hyperglycemia, they fail to significantly alter the course of diabetic complications [2]. Faced with the repeated failures of treatments due to insulin resistance and financial obstacles, it is necessary to look for new and more effective antidiabetic drugs with less side effects. Medicinal plants are natural chemical factories for human beings that produce active biochemical substances: alkaloids, essential oils, flavones, tannins, and make them available to humans who can use them for their health and meet their vital needs [3]. The vegetal kingdom then has become a target for the search for new drugs and biologically active compounds. Among the most used medicinal plants with great therapeutic interest are antidiabetic plants. The available literature shows that there are more than 400 plant species with antidiabetic activity [4]. In order to value medicinal plants by proving their scientific bases in traditional medicine, this study focuses on *flacourtia indica*, a species of Togolese flora. The present work was carried out in order to determine the phytochemical composition and the antioxidant, antibacterial and antidiabetic activities of the hydroethanolic and aqueous extracts of this plant.

MATERIALS AND METHODS

Plant materials

Flacourtia indica leaves were collected in September 2021 in Goumou Kope, a village located at 6.2N; 1.5E on the national n °2

roadside. The plant was identified in the botany and plant ecology laboratory of the department of botany of the Faculty of Sciences of the University of Lome in Togo. The collected leaves were washed and then dried at laboratory temperature for two weeks before being reduced to powder using an electric grinder. The powder obtained was kept for further uses.

Animals

Male and female wistar rats were used for the experiments in the present study. The rats are maintained under normal laboratory conditions and have free access to water and food throughout the experiment. The tests are carried out in accordance with ethical values and a license has been obtained from the ethics committee of the local institute. Besides, the general care of the experimental animals used for this study was done in compliance with the Animal Welfare Act.

Preparation of extracts

Extraction was made by maceration of 100 g of powder in 1L of solvent (distilled water for 24 h and a 20/80 hydroethanolic solution for 72 h). Mixtures were stirred intermittently. After filtration on cotton and then on Wattman No.4 paper, the filtrate obtained was evaporated using a rotary evaporator at 45 °C in order to obtain the dry extracts. The extracts obtained were then stored at 4 °C for later use. The extraction yield was calculated using the following formula:
 $R = (M_{ex}/M_p) \times 100$

R is the extraction yield;

M_{ex} is the mass of extract obtained;

M_p is the mass of powder used.

Phytochemical screening

For phytochemical screening, alkaloids, flavonoids, anthocyanins, anthraquinones, saponosides, tannins, terpenes, terpenoids, and

gallic tannins were sought by different methods for determining the major chemical groups based on coloring and/or precipitation tests.

Determination of polyphenols

The dosage of total polyphenols was based on the quantification of the total concentration of hydroxyl groups present in the extract and was determined by spectrophotometry according to the colorimetric method using the Folin-Ciocalteu reagent.

The protocol was based on the one described by Ali-Rachedi *et al.* [5] with a few modifications. In short, in test tubes, a volume of 200 μ l of each extract was added, with a mixture of 1 ml of Folin-Ciocalteu reagent diluted 10 times, and 800 μ l of a sodium carbonate solution at 7.5 %. The tubes are shaken and stored for 30 min away from light at room temperature. The absorbance was read using a spectrophotometer at 765 nm.

A calibration curve was produced in parallel under the same operating conditions using gallic acid at different concentrations (0 to 1000 μ g/ml).

Determination of total flavonoids

The quantification of flavonoids was carried out by a method based on the formation of a very stable complex between aluminum chloride and the oxygen atoms present on carbons 4 and 5 of the flavonoids [6].

The protocol used in this study was based on the protocol described by Ali-Rachedi *et al.* [5] with some modifications.

In a test tube 1 ml of extract was introduced, 2 ml of distilled water, then 300 μ l of 5% NaNO₂ were added. After 5 min, 200 μ l of 10% AlCl₃ were added, and the medium was mixed vigorously. After 6 min, a volume of 2 ml of ¹N Na₂CO₃ was added to the medium. The absorbance was read immediately at 510 nm against white color. An ethanol solution of quercetin was prepared. Child solutions prepared from the stock solution at different concentrations between 50 and 600 μ g/ml allowed the calibration curve to be plotted.

Antioxidant activity evaluation

DPPH reduction test

The antioxidant activity was evaluated by trapping free radicals. The DPPH radical that is a free stable radical at room temperature and soluble in ethanol, was used to determine the anti-radical potential of the extracts according to the method described by Agbodan *et al.* [7].

An ethanolic solution of DPPH was prepared and stored away from light. In test tubes containing 100 μ l of an ethanolic solution of each extract tested at different concentrations (20 to 150 μ l/mg), a volume of 2 ml of the DPPH solution was added. The mixture was homogenized by a vortex mixer and it was left in the dark. Quercetin used as a reference antioxidant, was also tested at different concentrations (10 μ l g/ml to 60 μ l g/ml).

After 30 min of incubation in the dark, the absorbance was read on a spectrophotometer at 517 nm using ethanol as a blank. Three tests were carried out for each concentration. The percentage inhibition (PI) of free radicals DPPH was calculated according to the formula:

$$PI (\%) = ((A_0 - A_1) / A_0) \times 100$$

-A₀: absorbance of DPPH•

-A₁: absorbance after addition of the products to be tested at a given concentration.

The required sample concentration so as to neutralize 50% of free radicals (IC₅₀) was determined graphically by linear regression.

Reduction of ferric ion (FRAP) test

The FRAP test is used to determine the total antioxidant potential of extracts. In the test, the electron donor capacity of the antioxidant is measured by the change in absorbance at 593 nm when a blue-colored ferrous tripyridyltriazine complex (Fe²⁺TPTZ) is formed from a colorless oxidized Fe³⁺. Ferric Reducing Ability of Plasma (FRAP) uses a mixture of three solutions: 25 ml of acetate buffer

with a concentration of 300 mmol/l, 2.5 ml of ferric tripyridyltriazine complex solution (TPTZ-Fe³⁺) and 2.5 ml of chloride of iron at 20 mmol/l. At 1500 μ l of the FRAP reagent, 150 μ l of distilled water is added, then 50 μ l of each extract. The mixture is incubated for 5 min then the antioxidant activity of the extracts is measured from the decrease in the absorbance of the ferrous tripyridyltriazine complex (TPTZ-Fe²⁺) at 593 nm [8].

Antibacterial activity

Extract sensitivity test

The extract sensitivity test was carried out by the diffusion method in agar wells, according to Afanyibo *et al.* [9] with some modifications. This was a presumptive test that could identify active extracts from a high concentration.

The microbial suspensions used were equal to 0.5 Mac Farland ($\approx 10^8$ UFC/ml). They were quantified by measuring the turbidity with a densitometer. The inoculum was introduced into culture media. The quality of these media was evaluated by sterility and fertility tests before using them. After seeding the medium, 6 mm diameter wells were made using a sterile hollow punch concentrically in the agar. Each well was flooded with 50 μ l of extract at a concentration of 200 mg/ml. Sterile distilled water was used instead of extract for negative controls. After 30 min of pre-diffusion at laboratory temperature, the Petri dishes were incubated for 18 to 24 h at a temperature of 35 ± 2 $^{\circ}$ C for the bacteria and 25 ± 2 $^{\circ}$ C for the yeasts. The diameters of the microbial growth inhibition zones were measured using an electronic reading table. Extracts with an inhibition diameter ≥ 12 mm (including disk) were used for the determination of minimum inhibitory (MIC) and bactericidal (MBC) concentrations. The tests were carried out in triplicate.

Determination of the minimum inhibitory concentration MIC and the minimum bactericidal concentration MBC

The test for establishing the sensitivity curve was carried out for the extracts, which resulted in a diameter of growth inhibition ≥ 12 mm with the tests of sensitivity to the extracts previously carried out (presumptive test). This investigation was carried out by the microplate dilution method. From the stock solution of 200 mg/ml of extract, a series of successive dilutions of a range of 6.5 to 100 mg/ml was carried out in Mueller Hinton Broth (MHB). The tubes were inoculated with 50 μ l of a microbial suspension at CFU/ml. Quality Control was done with MHB (not inoculated). MHB broth was used as a positive control to facilitate reading. MHB broth and extracts were used as a negative control. The tests were carried out under the control of the Microbiological Safety Station (PSM). The preparations were covered with parafilm and then incubated at the appropriate temperature for 24 h. After incubation, the tubes were observed with the naked eye.

The presence of turbidity or deposit corresponded to the presence of microbial culture. The tube corresponding to the lowest concentration of extract for which no culture was observed represented the MIC of the extract on the strain tested. Then, from the MIC, 100 μ l are taken from the tubes which have not given any microbial growth visible to the naked eye, then inoculated onto Plate Count Agar (PCA) medium. Incubation was carried out at the appropriate temperature for 24 h. The lowest concentration for which no colonies were observed was considered to be the CMB or MFC of the extract on the strain tested. The antibiotic power of the extract on the microbial strain was determined by the CMB/CMI ratio. The tests were carried out in triplicate.

Antidiabetic activity

Induction of experimental diabetes

After fasting for twelve hours, the induction of diabetes in diabetic groups was carried out by intraperitoneal injection of alloxan freshly prepared in a solution of physiological saline at a dose of 120 mg/kg of body weight. After injection, water bottles were replaced with bottles containing 5% glucose solution to overcome alloxan-induced hypoglycemia [10].

Animal treatment

After the onset of diabetes, the groups were kept in the same

conditions. The start of treatment with the hydroethanolic extract of *Flacourtia indica* began on the same day that the diabetes was installed. The total duration of treatment is 10 d.

Group 01: 5 normal rats fed by a standard diet for 10 d.

Group 02: 5 diabetic rats fed by a standard diet for 10 d.

Group 03: 5 diabetic rats fed by a standard diet and treated with 250 mg/Kg p. c. of the hydroethanolic extract of *Flacourtia indica* for 10 d.

Group 04: 5 diabetic rats fed by a standard diet and treated with 500 mg/Kg p. c. of the hydroethanolic extract of *Flacourtia indica* for 10 d.

Group 05: 5 diabetic rats fed by a standard diet and treated with 250 mg/Kg b.w. of Metformin for 10 d.

Evolution of body weight

In order to determine the influence of the extract on the body weight and the growth of the rats, the evolution of the body weight of the normal and treated rats was periodically evaluated throughout the experiment. The body weight of the rats was measured each day using a scale in grams (g) and the variations in the body weight of the rats compared to the 1st day are expressed as a percentage (%) and calculated according to the following formula:

$$\text{Change in body weight (\%)} = (P_j - P_{j_0}) \times 100 / P_{j_0}$$

P_{j_0} : body weight on the 1st day;

P_j : body weight on the D-Day.

Blood glucose determination

In order to determine the influence of the extract on the blood glucose of the rats during the period of the manipulations, four (4) glycaemia were measured. The first is carried out with the aim of checking the onset of diabetes in the groups treated with alloxan, taking as a reference normal blood sugar levels (before the start of treatment). After this blood glucose measurement, 3 others were measured on the 4th, 7th, and 10th day using the ACCU-CHEK Active strip glucometer.

Statistical analyzes

The results of the various tests were processed using Excel 2013 and GraphPad Prism 8.2.263 software and then expressed as a mean±SEM (Standard Error of the Mean).

RESULTS

Phytochemical screening

Phytochemical screening of aqueous and hydroethanolic extracts of *Flacourtia indica* leaves revealed the presence of tannins, flavonoids, saponosides, terpenoids, and anthraquinones. However, the absence of alkaloids was observed in two extracts (table 1).

Table 1: Phytochemical screening results

Investigated compounds	Aqueous extracts	Hydroethanolic extracts
Alcaloids	-	-
Tannins	+	+
Gallic tannins	+	+
Flavonoids	+	+
Anthocyanins	+	+
Saponins	+	+
Anthraquinones	+	+
Terpenoids	+	+
Terpenes	+	+

+: existence-: absence

Quantitative tests

Polyphenols

The total phenol contents of the hydroethanolic and aqueous extracts of *Flacourtia indica* were determined from the ($y = 0.011173x + 0.022972$; $R^2 = 0.98913$) linear regression of the gallic acid calibration curve. These contents estimated by the folin-ciocalteu method for each extract were reported in milligram equivalent of gallic acid per gram of dry extract. These contents are shown in fig. 1. It should be noted that the hydroethanolic extract contained more total phenols than the aqueous extract. These values proved to be 186.46 ± 0.308 mg Eq AG/g and 173.37 ± 0.503 mg Eq FA/g and mg Eq FA/g, respectively for the hydroethanolic extract and for the aqueous extract.

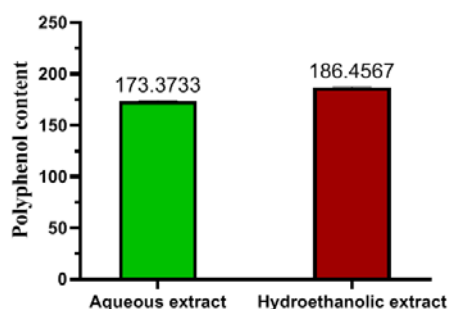


Fig. 1: Polyphenol content

Total flavonoids

The results obtained after the determination of total flavonoids for each *Flacourtia indica* extract using the aluminum trichloride method and determined from the ($y = 0.00066357x + 0.033117$; $R^2 = 0.97753$) linear regression line of the quercetin calibration curve were reported in milligram equivalent of quercetin per gram of dry extract (mg EqQ/g. ES). The results from this assay are shown in fig. 2. It was shown that the hydroethanolic extract was more concentrated in total flavonoids than the aqueous extract. These contents proved to be 464.14 ± 17.043 mgEqQ/g. ES and 393.19 ± 19.142 mgEqQ/g. ES and respectively for the hydroethanolic extract and for the aqueous extract.

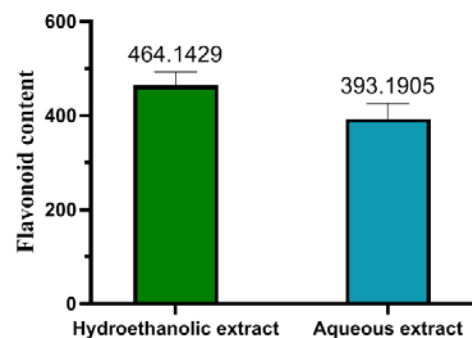


Fig. 2: Flavonoid content

Evaluation of the antioxidant activity of *Flacourtia indica* extracts

DPPH reduction test

The reducing power of the 2,2'-diphenyl-1-picrylhydrazyl radical (DPPH) of hydroethanolic and aqueous extracts of *Flacourtia indica* is determined from the linear regression line ($y = -0.0053973x + 0.79431$; $R^2 = 0.99726$) of the quercetin calibration curve.

For a better comparison of the antioxidant activities of the extracts, the values of the concentrations were determined in order to neutralize 50% of the free radicals (IC_{50}) from the curves of the percentages of inhibition of the radical according to the concentrations of each extract. The results obtained were reported in fig. 3. These values turned out to be 110.22 $\mu\text{g/ml}$ and 138.07 $\mu\text{g/ml}$, respectively for the hydroethanolic extract and the aqueous extract. Furthermore, the quercetin used as a reference molecule had an IC_{50} of 43.29 $\mu\text{g/ml}$.

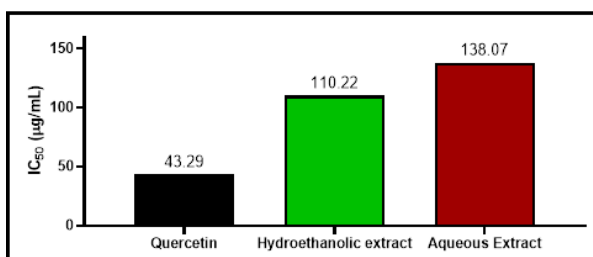


Fig. 3: DPPH radical reduction capacity of extracts

FRAP test (ferric ion reducing activity)

The presence of an electron donor antioxidant in a reaction mixture containing TPTZ leads to the reduction of ferric ions to ferrous ions. Thus, a modification of the coloring of the mixture was observed. In

order to determine the quantity of ferrous ions formed, the absorbance was measured at 593 nm. The reducing power of ferric ions in our extracts was determined from linear regression ($y = 0.0005280x - 0.0005346$). The results were expressed in $\mu\text{mol/mg}$ of dry extract as illustrated in fig. 4. It should be noted that the two extracts showed an appreciable reducing power. Its reducing powers were 3706 ± 12.124 and 3777 ± 9.238 $\mu\text{mol/ml}$, respectively for the hydroethanolic extract and the aqueous extract.

Antibacterial activity

Sensitivity test

The antibacterial power of hydroethanolic and aqueous extracts of *Flacourtia indica* leaves was evaluated *in vitro* by the method of diffusion in agar wells. This antibacterial power was estimated in terms of the diameter of the zone of inhibition around the discs containing the extract to be tested against the five (5) bacterial strains after 24 h of incubation. The results of the inhibition diameters were provided in table 2. For this test, we only took into account extracts with an inhibition diameter greater than or equal to 12 mm as being active against the bacterial strain tested. Thus, only *staphylococcus aureus* was susceptible to extracts.

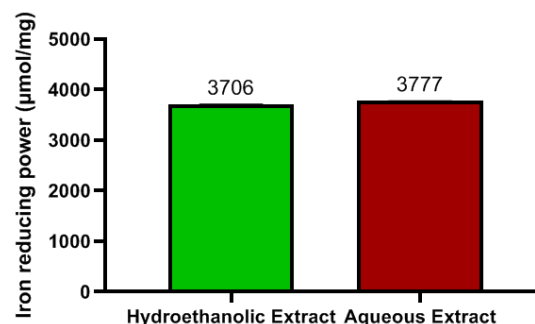


Fig. 4: Iron reducing power ($\mu\text{mol/mg}$)

Table 2: Inhibition diameter of extracts vis-à-vis *staphylococcus aureus*

Extracts	D1 (mm)	D2 (mm)	Diameter mean
Aqueous extract	17.23	18.2	17.71
Hydroethanolic extract	14.24	14.22	14.23

Determination of the minimum inhibitory concentration MIC and bactericidal MBC

Minimum inhibitory concentrations and minimum bactericidal concentrations of hydroethanolic and aqueous extracts of *Flacourtia indica* on *Staphylococcus aureus* were identified by the microplate dilution method. The MIC value was 6.25 mg/ml for both the hydroethanolic extract and the aqueous extract. In addition, the MBC values were respectively 25 mg/ml and 50 mg/ml for the aqueous and hydroethanolic extracts.

Antidiabetic activity

Body weight evolution

The evolution of the body weight of the rats during the ten days of treatment was recorded in fig. 5. The analysis of this fig. revealed that the body weight of normal rats increased throughout the experiment. For untreated diabetic rats, there was a weight gain until the 3rd day then a decrease in the body weight of the rats from the 4th day. This decrease became very significant compared to their initial weight at the end of the experiment ($P = 0.001907$). However, for diabetic rats receiving the extract at a dose of 250 mg/kg b.w. and those receiving the reference drug at a dose of 250 mg/kg b.w., a slight decrease was observed in the weight of the rats between the 4th and the 6th day after diabetes induction. From the 6th day, normal growth of the rats was noted until the end of the experiment. On the

other hand, for the rats of the diabetic group treated with the extract at a dose of 500 mg/kg b.w., a drop in the body weight of the rats was observed on the 3rd day, then a constant increase until the end of the test.

Blood glucose level evolution in rats

The evolution of blood glucose levels of normal and diabetic rats during the ten days of experimentation is shown in fig. 6. The analysis of the results showed that the glycaemia of normal rats was almost constant. These values vary between 105.8 ± 3.839 and 113.8 ± 2.672 mg/dl during the ten days of experimentation. However, an increase in the blood glucose of the untreated diabetic rats compared to the blood glucose of the group of normal rats was noted as soon as the disease sets in. This increase was high and constant during the experiment and ranging from 345.2 ± 38.534 up to 514 ± 9.503 mg/dl. With regard to the two diabetic groups treated with the extract and the group of diabetic rats treated with the reference drug, a very significant decrease ($P = 0.0014$) was observed in the blood glucose of the diabetic rats from the 4th day of treatment. The significant antihyperglycemic effect observed in treated diabetic rats was constant throughout the experiment. On the tenth day of the experiment, the diabetic rats were treated both with the two doses of the hydroethanolic extract of *flacourtia indica* and with the reference drug, they regained their normal blood sugar levels.

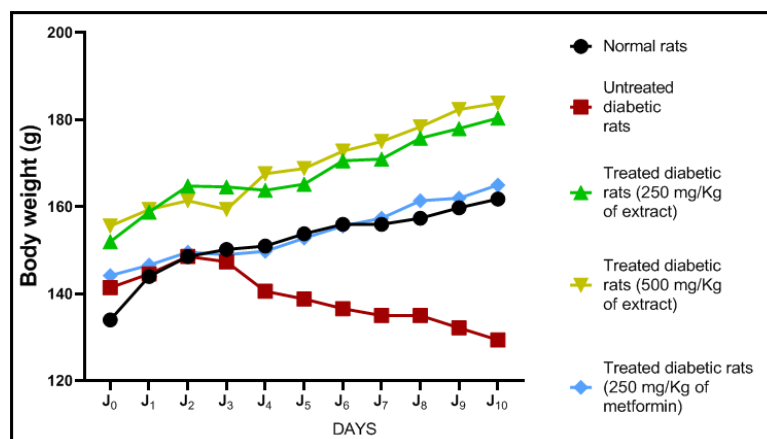


Fig. 5: Body weight evolution

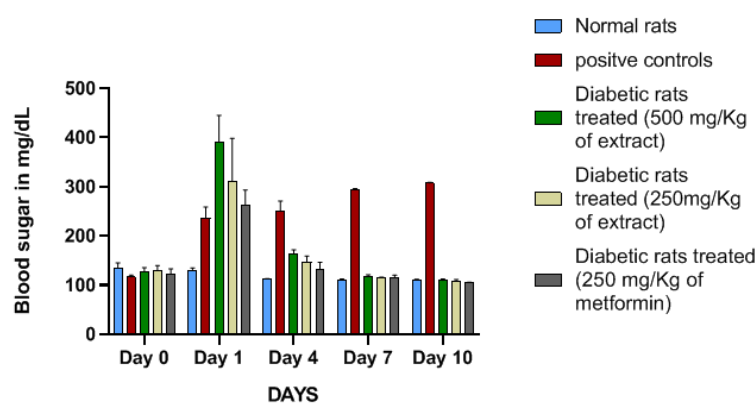


Fig. 6: Blood glucose level evolution in rats

DISCUSSION

The use of plant treatment as well as the search for new substances with biological activities, are one of the greatest scientific concerns. This can only be done by first making a call for phytochemical analysis, which will identify the different chemical groups that exist in the plant extract.

The phytochemical screening carried out on the extracts of the leaves of *Flacourtia indica* shows the presence of flavonoids, tannins, saponins, and terpenoids. However, there is the absence of alkaloids in the extracts, while Tyagi *et al.* [11] and Singh *et al.* [12] reported the presence of alkaloids in their studies. This difference highlights the variability of the composition in large chemical groups of plants belonging to the same species and could be linked to several factors, namely soil and climatic factors. The solvent and the extraction technique are also two factors that can explain this difference. Indeed, these two authors made a methanolic and hot extraction. A plant which is rich in phenolic compounds such as tannins, flavonoids, and saponins has antioxidant, antimicrobial, antifungal, and antidiabetic properties [13]. The presence of these different chemical groups in the leaves of *Flacourtia indica* would therefore justify its traditional use.

The results of the dosage of phenolic compounds (polyphenols and total flavonoids) showed that the hydroethanolic extract contains more polyphenols than the aqueous extract. The polyphenol concentration of an extract would be related to the nature of the extraction solvent used. Indeed, some researchers [14, 15] have found in their work that an ethanol-water combination allows better extraction of polyphenols. Furthermore, Naczka and Shahidi [16] reported that solvent polarity plays a key role in increasing the solubility of phenolic compounds. The results obtained in this study

are far superior to those found by Tyagi *et al.* [11] who worked on the Indian species. This difference in the contents could be explained by the environmental and climatic conditions and the collection period. Likewise, the experimental conditions, as well as the nature of the solvent, are important factors which could explain this difference.

The results from the DPPH• radical anti-radical test showed that the extracts have good anti-radical power. However, quercetin, used as a reference molecule, showed greater activity. Also, the hydroethanolic extract showed a higher potency compared to the aqueous extract. These differences observed on the one hand between the extracts and the quercetin could be justified by the purity of the quercetin and on the other hand, between the two extracts could be explained by the fact that the water-ethanol mixture better concentrated the phenolic compounds. Indeed, it is shown in the literature that polyphenols and flavonoids were major contributors to the antioxidant property of plants [15]. Studies have shown that antioxidant activity is linked to the concentration of phenolic compounds, so the higher their concentration, the greater the activity [17]. These results confirm those of Tyagi *et al.* [11] who had already reported good antioxidant activity of *Flacourtia indica* leaves.

In addition, the evaluation of the reducing power of ferric ions (FRAP) of the hydroethanolic and aqueous extracts of the leaves of *Flacourtia indica* showed that the aqueous extract as well as the hydroethanolic extract have a good capacity of reducing ferric ions. The high contents of flavonoids in our extracts would justify these good reductions of ferric ions observed in this work. Indeed, phenols and flavonoids play a key role in the chelation of transition metals involved in the Fenton reaction [18].

Furthermore, in order to support diabetic patients to overcome the problems of infections they are often confronted with, one of the objectives of this study is to evaluate the antibacterial activity of hydroethanolic and aqueous extracts of *Flacourtia indica* leaves on the *in vitro* growth of multi-resistant bacteria. This study shows that the extracts are only active on *Staphylococcus aureus* and are bacteriostatic on this bacterial strain. The phytochemical screening performed on the two extracts of this plant revealed the presence of chemical groups such as tannins, flavonoids, anthraquinones, and terpenoids with known antibacterial properties [19]. Tannins inhibit the growth of many microorganisms, including bacteria [20]. The antimicrobial activity of the extracts would be justified by the presence of quinones and derivatives [21]. The biological properties of these major chemical groups would justify the antimicrobial activities shown in this study and the traditional use of *Flacourtia indica* in the treatment of pathologies of bacterial origin.

Diabetes mellitus refers to the result of an imbalance in the metabolism of carbohydrates, lipids, and proteins, followed by defective insulin secretion or insulin action or both. Currently, its management by plant extract has become a trend. Several plant extracts are endowed with considerable hypoglycemic properties [22]. The antidiabetic activity of the hydroethanolic extract of the leaves of *Flacourtia indica* is evaluated *in vivo* in streptozotocin-induced diabetic rats by intraperitoneal injection of alloxan at 120 mg/kg of body weight. The injection of alloxan destroys the pancreatic β cells and causes a decrease in the release of insulin and therefore creates hyperglycemia in rats. This alloxan-induced hyperglycemia is characterized by severe loss of body weight and similar results are observed for untreated diabetic rats in this study. Treatment with the hydroethanolic extract of *Flacourtia indica* shows no significant difference ($P = 0.9842$) between the body weight of normal rats and that of treated diabetic rats. The extract allows recovery of the body weight of diabetic rats and, therefore, could protect the rats against weight loss associated with the disease of diabetes and can consequently ensure their normal growth. This recovery in body weight in rats could be due to the extract's ability to reduce hyperglycemia [23] and to the activation of protein synthesis [24].

The hydroethanolic extract of *Flacourtia indica* could therefore stimulate the pancreatic secretion of insulin, promoting the storage of lipids and triglycerides [25]. The study of the anti-hyperglycemic effect of the hydroethanolic extract of *Flacourtia indica* shows a decrease in the blood sugar levels of diabetic rats after 10 d of treatment. This anti-hyperglycemic effect may be due to the activation of insulin secretion from the beta cells of the islets of Langerhans or to the assurance of its release in active form [23]. The antidiabetic activity of our extract could be justified by the presence of flavonoids and tannins in this plant. Indeed, these secondary metabolites would have hypoglycemic properties [1]. Studies by Al-Taweel *et al.* [26] have shown that flavonoid compounds have

significant hypoglycemic activity in Swiss albino rats. Other studies have reported that tannins in general, can act on glucose in various ways, either by allowing better absorption of glucose in the peripheral tissues, by stimulating the secretion of insulin from the β cells of the pancreas or by decreasing the glycation of circulating proteins, in particular, glycated hemoglobin, a marker of long-term glycemic status in type 2 diabetes [27]. In addition, the antioxidant activity shown in these studies would improve the antioxidant defense mechanism and, therefore, could protect against diabetic complications.

CONCLUSION

The present study showed that hydroethanolic and aqueous extracts of the leaves of *Flacourtia indica* are significantly rich in secondary metabolites. The two extracts have a good antioxidant activity which is probably related to the phenolic compounds contained in these extracts. However, the hydroethanolic extract showed better antioxidant activity. The hydroethanolic extract of the leaves of *Flacourtia indica* reduced the blood glucose levels of diabetic rats (alloxan-induced) by more than 65% after ten days of treatment. Based on the obtained results in this research, further studies are needed in order to identify the toxicity of extracts of *Flacourtia indica* and the isolation as well as the characterization of the molecules that are responsible for these activities.

ACKNOWLEDGEMENT

The authors express their sincere gratitude to the *Laboratoires de Chimie Organique des Substances Naturelles (Lab COSNat)* and *Physiologie/Pharmacologie des Substances Naturelles* (laboratories of organic chemistry of natural substances and physiology/pharmacology of natural substances) for having served as a study environment.

FUNDING

Nil

AUTHORS CONTRIBUTIONS

All the authors have contributed equally.

CONFLICTS OF INTERESTS

All the authors have none to declare.

REFERENCES

1. Mangambu MJD, Mushagalusa KF, Kadima NJ. Contribution a l'etude photochimique de quelques plantes medicinales antidiabetiques de la ville de Bukavu et ses environs (Sud-Kivu, R. D. Congo). *J App Bioscience*. 2014;75(1):6211-20. doi: 10.4314/jab.v75i1.7.
2. Saravanan G, Ponnurugan P, Kumar GPS, Rajarajan T. Antidiabetic properties of S-allyl cysteine, a garlic component on streptozotocin-induced diabetes in rats. *J Appl Biomed*. 2009;7(3):151-9. doi: 10.32725/jab.2009.017.

3. Bakiri N, Bezzi M, Khelifi L, Khelifi Slaoui M. Enquete ethnobotanique d'une plante medicinale *Peganum harmala* L. dans la region de m'sila. *Rev Agric*. 2016;1:38-42.
4. Grover JK, Yadav S, Vats V. Medicinal plants of India with anti-diabetic potential. *J Ethnopharmacol*. 2002;81(1):81-100. doi: 10.1016/S0378-8741(02)00059-4, PMID 12020931.
5. Ali-Rachedi F, Meraghni S, Touaibia N, Mesbah S. Analyse quantitative des composés phénoliques d'une endémique algérienne *Scabiosa atropurpurea* sub. *Maritima* L. *Bull Soc Roy Sc de Liege*. 2018;87:13-21. doi: 10.25518/0037-9565.7398.
6. Doloking H, Mukhriani Ningsi S, Tahar N. Flavonoids: a review on extraction, identification, quantification, and antioxidant activity. *Ad-Dawaa'*. *Pharm Sci*. 2022;5(1):28-63. doi: 10.24252/djps.v5i1.29329.
7. Agbodan KML, Akpavi S, Agbodan KA, Kanda M, Amegnaglo KB, Adrou Aledji A. Description agromorphologique et détermination du potentiel antioxydant des variétés sous-utilisées et nouvellement introduites de maïs, manioc, niébe et piment dans la région Maritime-Est du Togo. *AJFAND*. 2020;20(3):15936-53. doi: 10.18697/ajfand.91.18625.
8. Nair VDP, Dairam A, Agbonon A, Arnason JT, Foster BC, Kanfer I. Investigation of the antioxidant activity of african potato (*Hypoxis hemerocallidea*). *J Agric Food Chem*. 2007;55(5):1707-11. doi: 10.1021/jf0619838, PMID 17295502.
9. Afanyibo YG, Anani K, Esseh K, Sadij Y, Idoh K, Koudouvo K. Antimicrobial activities of *syzygium aromaticum* (L.) merr. and *L. M. perry* (Myrtaceae) fruit extracts on six standard microorganisms and their clinical counterpart. *OALib*. 2018;5(12):1-13. doi: 10.4236/oalib.1104951.
10. Sharma VK, Kumar S, Patel HJ, Hugar S. Hypoglycemic activity of *ficus glomerata* in alloxan-induced diabetic rats. *Int J Pharm Sci Rev Res*. 2010;1(2):18-22.
11. Tyagi SN, Rakshit SA, Raghvendra SA, Patel BD. *In vitro* antioxidant activity of methanolic and aqueous extract of *flacourtia indica* merr. *Am Eurasian J Sci Res*. 2010;6(5):201-6.
12. Singh V, Singh M, Shukla S, Singh S, Mansoori MH, Kori MI. Antidiabetic effects of *flacourtia indica* merr. in streptozotocin induced diabetic rats. *Glob J Pharmacol*. 2011;5(3):147-52.
13. Manach C, Scalbert A, Morand C, Remesy C, Jimenez L. Polyphenols: food sources and bioavailability. *Am J Clin Nutr*. 2004;79(5):727-47. doi: 10.1093/ajcn/79.5.727, PMID 15113710.
14. Katalinic V, Mozina SS, Skroza D, Generalic I, Abramovic H, Milos M. Polyphenolic profile, antioxidant properties and antimicrobial activity of grape skin extracts of 14 *vitis vinifera* varieties grown in dalmatia (Croatia). *Food Chem*. 2010;119(2):715-23. doi: 10.1016/j.foodchem.2009.07.019.
15. Koffi E, Sea T, Dodehe Y, Soro S. Effect of solvent type on extraction of polyphenols from twenty-three Ivorian plants. *J Anim Plant Sci*. 2010;5(3):550-8.
16. Naczki M, Shahidi F. Extraction and analysis of phenolics in food. *J Chromatogr A*. 2004;1054(1-2):95-111. doi: 10.1016/S0021-9673(04)01409-8, PMID 15553136.
17. Masuku NP, Unuofin JO, Lebelo SL. Phytochemical content, antioxidant activities and androgenic properties of four South African medicinal plants. *J Herb Med Pharmacol*. 2020;9(3):245-56. doi: 10.34172/jhp.2020.32.
18. Valko M, Leibfritz D, Moncol J, Cronin MT, Mazur M, Telser J. Free radicals and antioxidants in normal physiological functions and human disease. *Int J Biochem Cell Biol*. 2007;39(1):44-84. doi: 10.1016/j.biocel.2006.07.001. PMID 16978905.
19. Daglia M. Polyphenols as antimicrobial agents. *Curr Opin Biotechnol*. 2012;23(2):174-81. doi: 10.1016/j.copbio.2011.08.007. PMID 21925860.
20. Sepulveda L, Ascacio A, Rodriguez Herrera R, Aguilera Carbo A, Aguilar CN. ChemInform abstract: ellagic acid: biological properties and biotechnological development for production processes. *Chem Inform*. 2012;43(50). doi: 10.1002/chin.201250260.
21. Mahamane IIA, Bakasso S, Alio SA, Ilagouma AT, Yaou C, Moctar C. Phytochemical investigation and antimicrobial activity of six plants used in children's ailments treatment in Niger. *J Dis Med Plants*. 2021;6(4):92-7. doi: 10.11648/j.jdmp.20200604.15.
22. Coelho GDP, Martins VS, do Amaral LV, Novaes RD, Sarandy MM, Gonçalves RV. Applicability of isolates and fractions of plant extracts in murine models in type II diabetes: a systematic review. *Evid Based Complement Alternat Med*. 2016;2016:3537163. doi: 10.1155/2016/3537163, PMID 27843476.
23. Stanely P, Prince M, Menon VP. Hypoglycaemic and other related actions of *Tinospora cordifolia* roots in alloxan-induced diabetic rats. *J Ethnopharmacol*. 2000;70(1):9-15. doi: 10.1016/S0378-8741(99)00136-1, PMID 10720784.
24. Gandhi GR, Ignacimuthu S, Paulraj MG. Hypoglycemic and β -cells regenerative effects of *Aegle marmelos* (L.) corr. bark extract in streptozotocin-induced diabetic rats. *Food Chem Toxicol*. 2012;50(5):1667-74. doi: 10.1016/j.fct.2012.01.030. PMID 22310238.
25. Kim SH, Hyun SH, Choung SY. Anti-diabetic effect of cinnamon extract on blood glucose in db/db mice. *J Ethnopharmacol*. 2006;104(1-2):119-23. doi: 10.1016/j.jep.2005.08.059. PMID 16213119.
26. Al-Taweel AM, Perveen S, Fawzy GA, Alqasoumi SI, El Tahir KEH. New flavane gallates isolated from the leaves of *plicosepalus curviflorus* and their hypoglycemic activity. *Fitoterapia*. 2012;83(8):1610-5. doi: 10.1016/j.fitote.2012.09.010. PMID 22986293.
27. Masunda TA, Mbala MB, Kayembe SJ, Longoma BF, Ngbolua KN, Tshibangu DST. Activite anti-hyperglycémique et antiradicalaire des extraits des fruits de *raphia gentiliana* de wild. *Areaceae Int J Biol Chem Sci*. 2014;8(6):2441-51. doi: 10.4314/ijbcs.v8i6.7.