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Bioactivity-guided fractionation and identification of bioactive compounds from *Corchorus olitorius* L. leaf powders: effect on GLUT4 translocation

Assiéné Agamou Julien Armel^{1*} , Assiéné Oyong Damase Serge² and Ngah Esther³

Abstract

The management of type 2 diabetes through powdered leaves of *Corchorus olitorius*, commonly consumed and rich in bioactive compounds, is increasingly observed within populations. The objective of this study was to identify the bioactive compounds in extracts from powdered leaves of *C. olitorius* that enhance GLUT4 translocation. To this end, GLUT4 translocation activities at concentrations of 50, 100, and 200 µg/ml, as well as the identification of chemical compounds via LC/MS–MS from extracts obtained using a solvent mixture (Water/Ethanol: 3/7 v/v) after harvesting and grinding the leaves of *C. olitorius*, were conducted. The chemical structures, concentrations, and activity durations of the compounds of interest were determined following a bio-guided fractionation of the most active extracts. The results indicate that the extract at 200 µg/ml significantly increases the amount of GLUT4 translocated ($86.39 \pm 2.38\%$ Insulin on average) and contains predominant bioactive compounds belonging to the flavonoid family, characterized by chemical structures of quercetin (quercetin 3-O-(6"-acetyl-glucoside)) and quinic acid (3,4-dicaffeoylquinic acid and 3,5-dicaffeoylquinic acid). The activity duration and concentration of the compounds have a highly significant influence on the increase in GLUT4 levels. Notably, 3,5-dicaffeoylquinic acid exhibits the most substantial increase in GLUT4 levels ($116.00 \pm 3.00\%$ Insulin) after only 10 min of activity at a concentration of 50 µg/ml. These compounds may contribute to a rapid reduction in postprandial blood glucose levels in diabetic patients who regularly consume *C. olitorius* leaf powder.

Keywords *C. olitorius* leaf powders, Bioactive compounds, GLUT4 translocation, Bioactivity-guided fractionation, 3,5-dicaffeoylquinic acid

Introduction

Diabetes is a chronic metabolic disease characterized by persistent hyperglycemia, resulting either from a defect in insulin secretion or from an inability of cells to effectively utilize insulin [1]. Current treatments for type 2 diabetes include oral hypoglycemic agents (metformin, sulfonylureas, acarbose, etc.) and insulin therapy (insulin injections, insulin pens, insulin pumps, etc.), but these options are not

without limitations (costly, difficult to access, burdensome, etc.) and side effects (gastrointestinal disturbances, loss of appetite, fatigue and weakness, allergic reactions, etc.) [2]. Therefore, it is crucial to explore effective and safe therapeutic alternatives to improve the management of this disease. The search for commonly consumed food matrices rich in bioactive compounds is increasingly observed and recommended by many therapists within African populations, where numerous claims report that these matrices treat various chronic metabolic diseases [3].

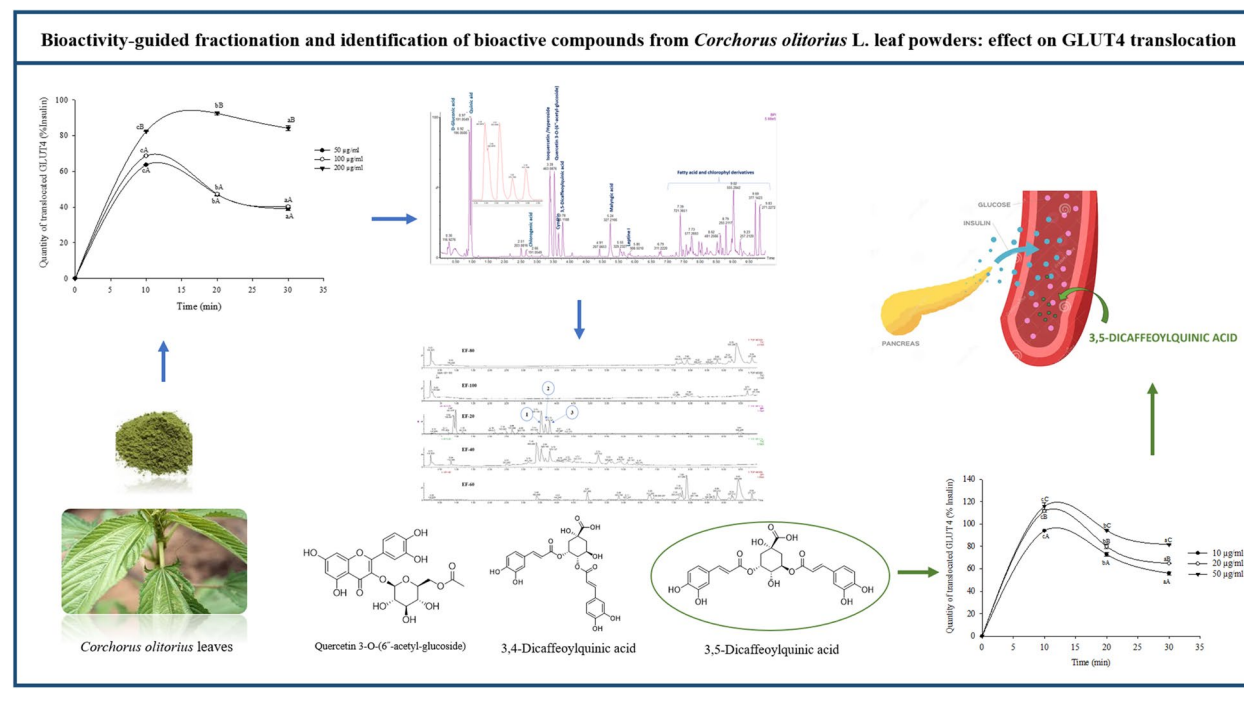
Corchorus olitorius (Malvaceae), also known as jute mallow, is commonly consumed in Asia and Africa (Cameroon,

*Correspondence:

Assiéné Agamou Julien Armel
assieneaja@yahoo.fr

Full list of author information is available at the end of the article

Graphical Abstract



Egypt, Ivory Coast, Nigeria, Benin) for its viscosity, nutritional value (proteins, sugars, fibers, minerals), and multiple health benefits (treatment of diseases such as dysentery, malaria, fever, etc.) [4]. This leafy vegetable is rich in bioactive compounds such as polyphenols, flavonoids, tannins, and saponins, which possess antioxidant as well as antihyperglycemic properties [5]. It is processed into powder and recommended as a functional food by many therapists in the fight against chronic diseases such as diabetes (Unpublished work). However, very few studies have focused on the ability of extracts and bioactive compounds from this plant to enhance the translocation of GLUT4, a key glucose transporter in blood glucose regulation.

Glucose transporter type 4 (GLUT4) plays a crucial role in glucose metabolism by facilitating its transport from the blood to muscle and fat cells, where it is used to produce energy [6]. When glucose enters the bloodstream after a meal, a precise concentration of insulin released (50 to 20 units/ml, or 0.1735 to 0.694 µg/ml) by the pancreas binds to its receptors on muscle and fat cells, triggering a cascade of cellular signaling [7]. This results in the translocation of GLUT4 to the cell membrane, meaning the movement of GLUT4 storage vesicles to the plasma membrane of the cell. Once GLUT4 is sufficiently present on the cell surface, it allows glucose to pass from the blood into the cell, thereby reducing blood glucose levels (glycemia) [8]. This reduction

in glycemia begins almost immediately after food consumption. However, it is typically after about thirty minutes that a significant decrease in glucose is generally observed to prevent glycemic spikes [7]. Glycemia is regulated approximately two hours later in a normal individual. However, in diabetic patients, who are subject to chronic insulin resistance (insensitivity or obstruction of insulin receptors), this mechanism is often impaired [6]. This quantitatively reduces the presence of GLUT4 at the cell membranes, thereby hindering the regulation of glycemia over time; this can last for several hours or even days [9].

From the above, it is evident that the regulation of blood glucose levels is closely related to the concentration of insulin released, the amount of GLUT4 translocated, and the timing involved. Several authors have reported that molecules other than insulin can induce GLUT4 translocation, including polyphenols such as procyanidin, resveratrol, anthocyanins [10, 11], curcumin, gallic acid, catechin, epicatechin, epigallocatechin, epigallocatechin gallate, and gallic catechin gallate [12]. Therefore, it is reasonable to consider that the intake of one or more bioactive compounds derived from medicinal plants may partially or completely substitute for insulin. This list of bioactive compounds is not exhaustive, given the limited volume of scientific research on the GLUT4 translocation activity of powdered leaves of *C. olerarius*. The search for such compounds remains a

major challenge for many researchers. Some have developed increasingly effective methods for obtaining these compounds. Among these methods, bioactivity-guided fractionation is a technique for exploring the therapeutic potential of natural extracts. It not only allows for the identification of compounds of interest but also deepens the understanding of the interactions between compounds and biological systems [13]. To this end, the present study aimed to identify the bioactive compounds from the extracts of *C. olitorius* leaf powder that enhance GLUT4 translocation through bioactivity-guided fractionation.

Material and methods

Sampling of *C. olitorius* leaves

The commonly consumed leaves of *C. olitorius* were harvested in October 2024, early in the morning, from fields in the Dibamba municipality, Sanaga Maritime Department, Littoral Region. They were identified by the Cameroonian herbarium:

- *C. olitorius*: The specimen was identified by comparison with the herbarium material of Westphal collector 10077 from herbarium collection specimen number 44871 SRFCam.

Powder production

The leaf powders were produced according to the requirements of therapists involved in the development of functional foods for the management of chronic diseases [3]. Fresh leaves were cleaned, washed, and cut into pieces approximately 5 mm in size. They were dried at 35 °C for 10 h in a Stockli dehydrator (Dorrex, France) and then ground into powders (particle size less than 500 µm) using a Philips blender (Model HR2058/00, 450 W, 220–240 V ~ 50–60 Hz, manufactured in China). The obtained powders were stored in an opaque glass container away from light at ambient temperature.

Chemical reagents

Chemicals including ethanol, methanol, acetonitrile, formic acid, quercetin 3-O-(6"-acetylglucoside), 3,4-dicaffeoylquinic acid, 3,5-dicaffeoylquinic acid and CHO-K1 cells were purchased from Glentham Life Sciences (UK), while human insulin was obtained from Sigma Aldrich, Schnelldorf (Germany).

Preparation of *C. olitorius* leaf powder extracts

Twenty-two milliliters (22 ml) of a solvent mixture Water/Ethanol 3:7 (v/v) were added to 1 g of powder, and the mixture was subjected to ultrasonic bath for 30 min, followed by shaking for 15 min. This process was repeated twice to maximize the extraction of secondary metabolites. The resulting

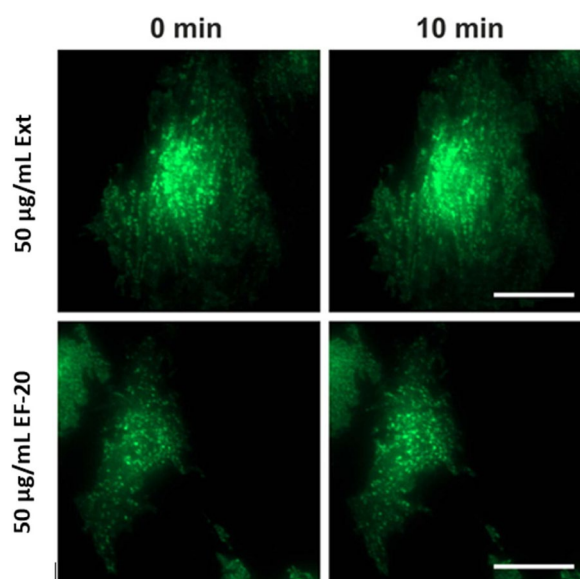
solution was centrifuged for 10 min at a speed of 13,400 revolutions per min, and the supernatant was collected, evaporated under reduced pressure, the freeze-drying.

Evaluation of GLUT4 translocation

GLUT4-myc-GFP cells were cultivated in 96-well imaging plates overnight to avoid light disturbances. The cell culture medium was aspirated, and after washing the cells with HBSS, it was replaced with HBSS for 3 h. The cells were incubated with insulin or the dissolved samples at different concentrations (extracts, fractions, and pure compounds) in KRPH buffer and imaged on an Olympus IX-81 inverted microscope in a total internal reflection (TIR) configuration via a Plan-Apochromat Olympus 60 NA = 1.49 objective lens. The 96-well plates were placed on an x-y stage (CMR-STG-MHIX2 motorized table). Scanning of larger areas was supported by an automated focus maintenance system guided by laser (ZDC2). Emission at 488 nm from a diode laser was used to image the fluorescence of the green fluorescent protein (GFP). After appropriate filtering, the fluorescence signal was recorded using an Orca-R2 CCD camera, allowing for the deduction of transporter percentages. The increase in GLUT4 (ΔS) is the percentage of signal obtained with the substances (extracts, fractions, or compounds) (S_w) minus the percentage of signal obtained without the substances (S_{w0}).

$$\Delta S = S_w - S_{w0}$$

Human insulin was used (100 nM) as the reference hormone (standard and positive control). The results obtained were expressed as a percentage of insulin (% Insulin). The following image illustrates the observed appearance.



UPLC-UV-ESI-TOF-MS analysis of extract and fraction

Aliquots of the extracts (1 ml each) were analyzed using UPLC/ESI-TOF-MS on a mass spectrometer coupled with an Acquity UPLC central system (Waters, Milford, MA, USA) equipped with a BEH C18 column (2 × 150 mm, 1.7 μm) (Waters, Manchester). Operated at a flow rate of 0.5 ml/min at 40 °C, the following gradient was used for chromatography: starting with a mixture (99/1, v/v, A/B) of acetonitrile and aqueous 0.1% of formic acid 0.1% in water (A) and acetonitrile (B), the B content was increased to 100% over 10 min. The scan time for the MSe (centroid) method was set to 0.2 s. Analyses were performed in negative ESI mode at high resolution using the following ion source parameters: capillary voltage +2.5 kV, sampling cone 20 V, source offset 40 V, source temperature 120 °C, desolvation temperature 450 °C, cone gas flow 2 L/h, nebulizer 6.5 bar, and desolvation gas 850 L/h. Data processing was performed using MassLynx 4.1 SCN 9.16 (Waters, Manchester) and the elemental composition tool to determine accurate mass. The structures of the compounds were determined using elemental compositions combined with ChemSpider, along with phytochemical studies.

Bio-guided fractionation of extracts

A mass of 50 mg of leaf extract was dissolved in 1 ml of water and suggested to ultrasonic bath for 5 min using 5 ml Falcon tubes. The mixture was centrifuged (table centrifuge, 4000 rpm) for 2 min, and the supernatant was collected. Fractionation was performed using elution systems of Water/Methanol: 80/20; 60/40; 40/60; 20/80; 0/100 v/v, through a Chromabond C18ec SPE column (1 g of matrix/5 ml) activated with 4 ml of 100% methanol (LC quality) and equilibrated with 6 ml of water. The five fractions obtained after elution were indexed as EF-20, EF-40, EF-60, EF-80, and EF-100, respectively. Five microliters (5 μl) of each fraction were reserved for LC/MS-MS analysis (see Sect. "UPLC-UV-ESI-TOF-MS analysis of extract and fraction") to identify the major compounds, while the remaining fractions after solvent evaporation were used for the evaluation of the ability to increase the percentage of GLUT4 translocation (see Sect. "Evaluation of GLUT4 translocation"). The most active fraction was considered for the further steps. Its major compounds were identified by the means of LC/MS analysis and purchased for bioassay. The bioassays on the major compounds were performed at three concentrations (10, 20, 50 μg/ml) and at different time points (10, 20, 30 min). The most active compound was isolate to clarify its structure by the means of 1D and 2D NMR analysis. A confirmation test of the biological activity of these compounds was performed.

Statistical analyses

Analyses were performed in triplicate ($n = 3$). Microsoft Excel 2016 was used for calculating means and standard deviations. SigmaPlot 2011 (Systat Software, Inc., 1735 Technology Drive, Suite 430, San Jose, CA 95110, USA) was used for graph plotting, and Statgraphics Centurion 15.2 (StatPoint Technologies, Inc., Warrenton, Virginia, USA) was used for multiple variance analysis (two factors) and for differences between means using Duncan's multiple range test at a significance level of $P < 0.05$.

Results and discussion

Effect of *C. olitorius* leaf powder extract on GLUT4 translocation and bioactive compounds identification

Figure 1 presents the biological activities of extracts from *C. olitorius* leaf powders on GLUT4 translocation. The results indicate that the amount of GLUT4 translocated varies significantly over time ($P = 0.0002$) while increasing with the concentration of the extracts ($P = 0.0000$). In this regard, an average increase of $71.57 \pm 2.38\%$ Insulin is observed during the first 10 min of the experiment. This average value decreases to $54.47 \pm 2.38\%$ Insulin after 30 min. Conversely, with extract concentrations ranging from 50 to 200 μg/ml, the amount of GLUT4 translocated varies from $49.86 \pm 2.38\%$ to $86.39 \pm 2.38\%$ Insulin, respectively. It is observed that the concentration of the extracts has the highest effect (75.53%) on the amount of translocated GLUT4 compared to time (17.72%). In this regard, the extract at a concentration of 200 μg/mL exhibited a higher average activity on GLUT4 of $86.39 \pm 2.38\%$ Insulin.

These results can be explained by the fact that the concentration of the bioactive compounds whose biological properties contribute to the movement of GLUT4 storage vesicles to the cell membrane in the extract increase with the concentration of *C. olitorius* leaf extract [12]. Indeed, the solvent mixture used (Water/Ethanol 3:7 v/v) allowed for a broader polarity of the solvent to extract a wide range of chemical compounds, including lipophilic compounds (fatty acids, terpenoids, etc.) and hydrophilic compounds (polyphenols, saponins, etc.). Similar results have been reported by numerous authors regarding chemical compounds extracted from plant matrices using solvent mixtures, which exhibit various biological activities [14, 15]. However, it is important to note that the increase in extract concentration enhances GLUT4 translocation activity simply because the chemical compounds present are at higher concentrations. It is therefore essential to more precisely identify the families of chemical compounds involved in this increase in the amount of GLUT4 observed in this study.

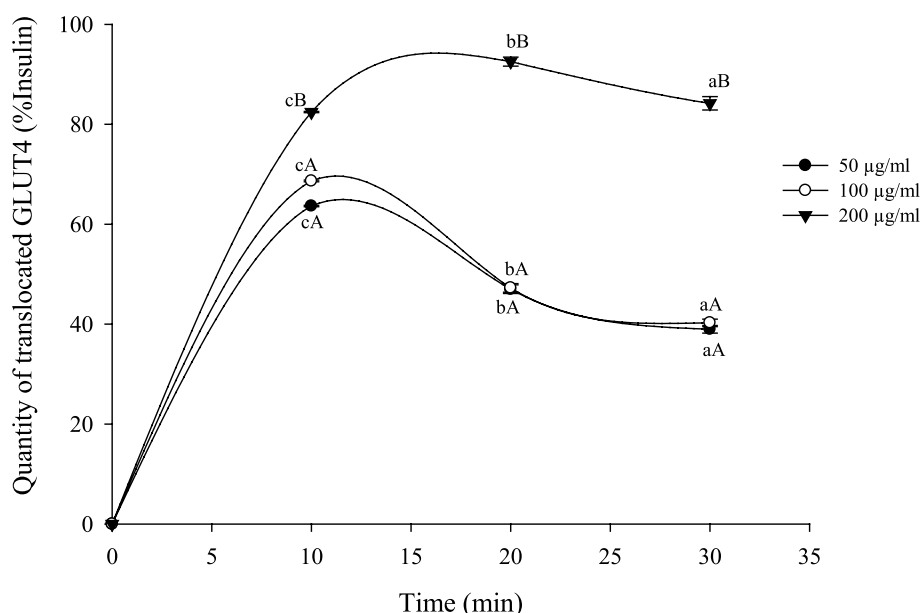


Fig. 1 Biological activities of extracts (Water/Ethanol 3:7 v/v) from *C. olitorius* leaf powder at different concentrations (50, 100, 200 µg/ml) on GLUT4 over time; means marked with different lowercase letters (time effect) and uppercase letters (extract concentration effect) indicate significant differences at a probability threshold of $P < 0.05$. The time effect: Sum of squares = 13.19%, $P = 0.0002$; Extract concentration effect: Sum of squares = 75.53%, $P = 0.0000$; Residual: Sum of squares = 11.26%

The results of the analysis of *C. olitorius* leaf powder extracts by LC/MS performed in negative mode are presented in Fig. 2. Several chromatographic peaks (Rt: retention time) and fragment masses (m/z) of corresponding compounds are observed. This allowed for the distinction of several families of major chemical compounds, including polyphenols such as chlorogenic acid (Rt = 2.66 min; m/z = 191.0549), 3,5-dicaffeoylquinic acid (Rt = 3.78 min; m/z = 515.1188); flavonoids such as isoquercetin (Rt = 3.39 min; m/z = 463.0876), quercetin 3-O-(6''-acetyl-glucoside) (Rt = 3.52 min; m/z = 505.1000); a peptide hormone such as leptin 1 (Rt = 5.85 min; m/z = 908.5010); organic acids such as D-gluconic acid (Rt = 0.92 min; m/z = 195.0500), quinic acid (Rt = 0.97 min; m/z = 191.0549), malonic acid (Rt = 5.24 min; m/z = 327.2166); fatty acids and chlorophyll derivatives ($7.39 \leq \text{Rt} \leq 9.83$ min; $271.2272 \leq \text{m/z} \leq 721.3661$). Similar results highlighting several of these compounds by LC/MS have been reported by numerous authors: chlorogenic acid [16], 3,5-dicaffeoylquinic acid [17, 18], isoquercetin [19], quercetin 3-O-(6''-acetyl-glucoside) and D-gluconic acid [20], quinic acid [21], malonic acid [22]. These families of compounds, particularly polyphenols and flavonoids, possess GLUT4 translocation activities [12]. However, at this stage of the study, it is not possible to determine which of these clearly identified compounds (Fig. 2) are responsible for the increase in the amount of translocated GLUT4. Therefore, a bio-guided

fractionation of the extract from *C. olitorius* leaf powders (ECOP), which exhibited a significant activity and close to that of insulin was performed.

Effect of ECOP fractions on GLUT4 translocation and bioactive compounds identification

Figure 3 presents the fractions obtained using the five solvent systems, namely EF-20, EF-40, EF-60, EF-80, and EF-100, along with their GLUT4 translocation activities over time. It is observed that, unlike time, only the ECOP fraction shows highly significant effects ($P = 0.0000$) and greater importance (96.65%) on the variations in the amount of translocated GLUT4. Three fractions exhibit positive effects on the amount of GLUT4, specifically EF-20, EF-40, and EF-60, with average values of 83.45 ± 3.87 ; 24.50 ± 3.87 ; and $6.08 \pm 3.87\%$ Insulin, respectively. The other two fractions, EF-80 and EF-100, display toxic effects on the amount of GLUT4, with average values of -60.13 ± 3.87 and $-79.94 \pm 3.87\%$ Insulin, respectively. However, it is important to note that the EF-20 fraction, composed of 80% water and 20% methanol, exhibits the highest GLUT4 translocation activity ($83.45 \pm 3.87\%$ Insulin).

These multiple variations in GLUT4 translocation activity are certainly due to the different identified chemical compounds and can be explained by previously mentioned reasons [12, 15]. However, the principle of varying solvent volumes used in the solvent mixture systems during the chromatographic separation of the

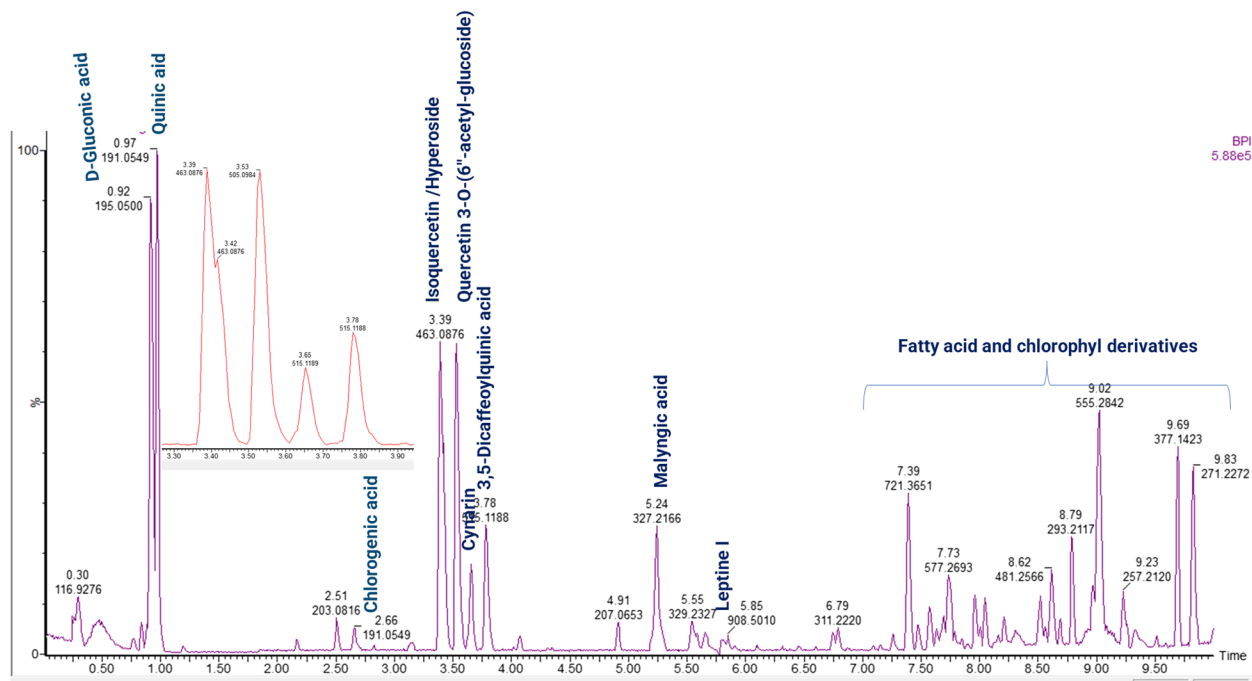


Fig. 2 UPLC/ESI-TOF-MS chromatogram of *C. olitorius* leaf extracts

extract allows for the elution of a wide range of chemical compounds, predominantly hydrophilic or highly polar in nature. It is important to note that as the volume of alcohol (methanol) increases, GLUT4 translocation activity decreases (EF-60) to the point of becoming toxic (EF-80, EF-100). This result can be explained by the fact that the compounds eluted with the desired activity are more concentrated in the fractions containing more

hydrophilic compounds (EF-20; EF-40), notably chlorogenic acid, 3,5-dicaffeoylquinic acid, isoquercetin, and quercetin 3-O-(6''-acetyl-glucoside). Similar results have been reported by several authors regarding the GLUT4 translocation activities of chlorogenic acid [8]. However, very few studies, if any, were focused on the GLUT4 translocation activity of isoquercetin, quercetin 3-O-(6''-acetyl-glucoside), and 3,5-dicaffeoylquinic acid

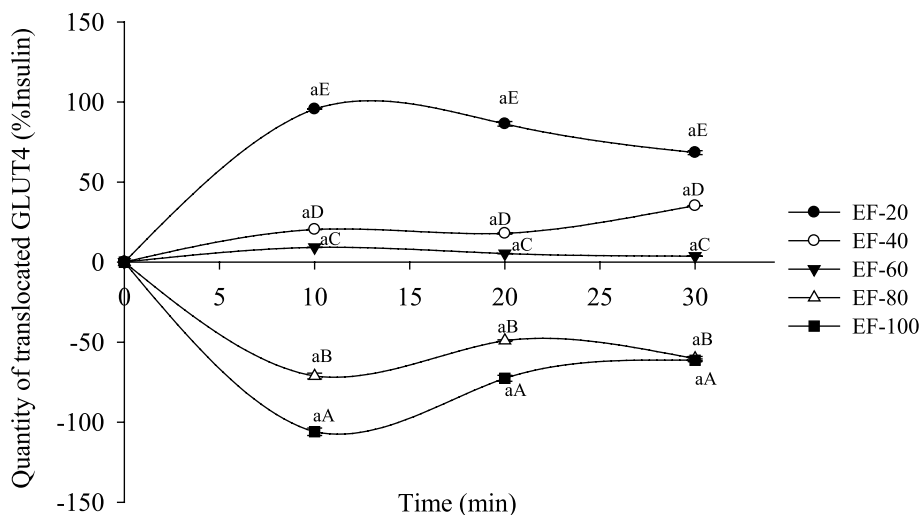


Fig. 3 Fractions from the extracts of *C. olitorius* leaf powders (ECOP) and their activities on GLUT4 over time (EF-20; EF-40; EF-60; EF-80; EF-100 = ECOP fractions obtained from the Water/Methanol system: 80/20; 60/40; 40/60; 20/80; 0/100 v/v, respectively); means marked with different lowercase letters (time effect) and uppercase letters (ECOP fraction effect) indicate significant differences at a probability threshold of $P < 0.05$; Time: Sum of squares = 0.37%, $P = 0.1201$; ECOP Fraction: Sum of squares = 96.48%, $P = 0.0000$; Residual: Sum of squares = 3.14%

isolated from *C. olitorius* leaf powders. Nonetheless, these compounds, specifically quercetin 3-O-(6"-acetyl-glucoside) and 3,5-dicaffeoylquinic acid, are identified as the major compounds responsible for the observed GLUT4 translocation activities in the ECOP fractions.

Figure 4 presents the LC/MS spectra of the five studied fractions (EF-20, EF-40, EF-60, EF-80, EF-100). It is observed that the EF-20 fraction contains three major compounds with retention times (Rt) and fragment masses (m/z) of Rt = 3.53 min, m/z = 505.1000 for the first compound; Rt = 3.79 min, m/z = 515.1210 for the second and third compounds, which are isomers. The mass spectra of these three compounds presented in Fig. 5 confirmed not only the exact mass of each compound but also its chemical structure. Indeed, the first compound, whose chemical structure consists of a flavonoid core (quercetin) linked to an acetylated glucoside group, has an exact mass of 505.0995 corresponding to quercetin 3-O-(6"-acetyl-glucoside). The second compound, with an exact mass of 515.1202 and a chemical structure consisting of a quinic core linked to two caffeoyl groups (caffeic acid) at positions 3 and 5, corresponds to 3,5-dicaffeoylquinic acid. The third compound, an isomer of 3,5-dicaffeoylquinic acid, differs by the position of the caffeoyl groups at position 4 and corresponds to 3,4-dicaffeoylquinic acid. These three flavonoids are powerful bioactive compounds known for their multiple biological activities: antioxidant, anti-inflammatory, antiviral, antidiabetic, etc. [17, 18, 23, 24]. However, their GLUT4 translocation

activities from extracts of *C. olitorius* leaf powders seem to be very little explored.

Effect of quercetin 3-O-(6"-acetyl-glucoside), 3,5-Dicaffeoylquinic acid, and 3,4-Dicaffeoylquinic acid on GLUT4 translocation activity

The chemical compounds (quercetin 3-O-(6"-acetyl-glucoside), 3,5-dicaffeoylquinic acid, and 3,4-dicaffeoylquinic acid), whose structures were previously determined, were purchased and used to evaluate their effect on GLUT4 translocation. Figure 6 presents the activities of these three compounds at different concentrations (10, 20, 50 µg/mL) on GLUT4 translocation over time (10, 20, 30 min). It is evident that regardless of the compound used, both time and concentration have highly significant effects ($P = 0.0000$) on variations in the amount of translocated GLUT4 (Fig. 6). The concentration of the compound is the most important factor to consider when examining the activity of quercetin 3-O-(6"-acetyl-glucoside) (76.45%) and 3,4-dicaffeoylquinic acid (72.74%). In contrast, for 3,5-dicaffeoylquinic acid, time is the most significant factor (73.64%) to consider.

These results indicate that as the concentration of the compound (quercetin 3-O-(6"-acetyl-glucoside) and 3,4-dicaffeoylquinic acid) increases, its activity expressed as a percentage of insulin tends to improve. Specifically, a concentration of 50 µg/mL of quercetin 3-O-(6"-acetyl-glucoside) increases the amount of translocated GLUT4 to 75.55%Insulin after just 10 min of activity (Fig. 6A). Similarly, a concentration of

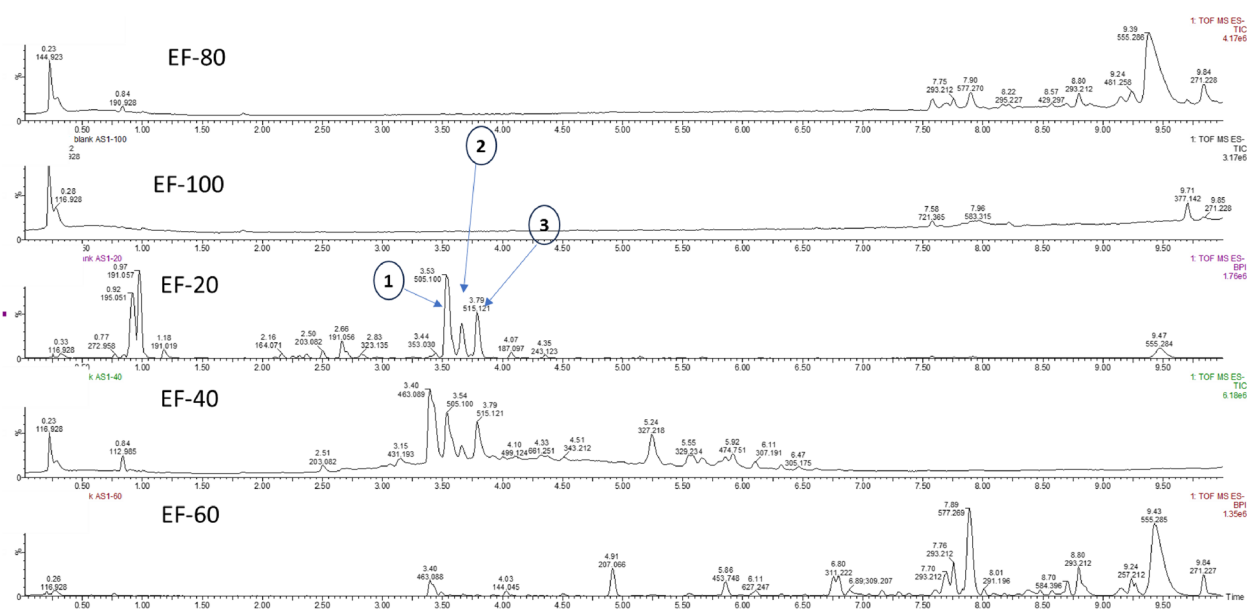


Fig. 4 UPLC-ESI-MS Chromatogram of fractions (EF-20; EF-40; EF-60; EF-80; EF-100) from the extract of *C. olitorius* leaf powder

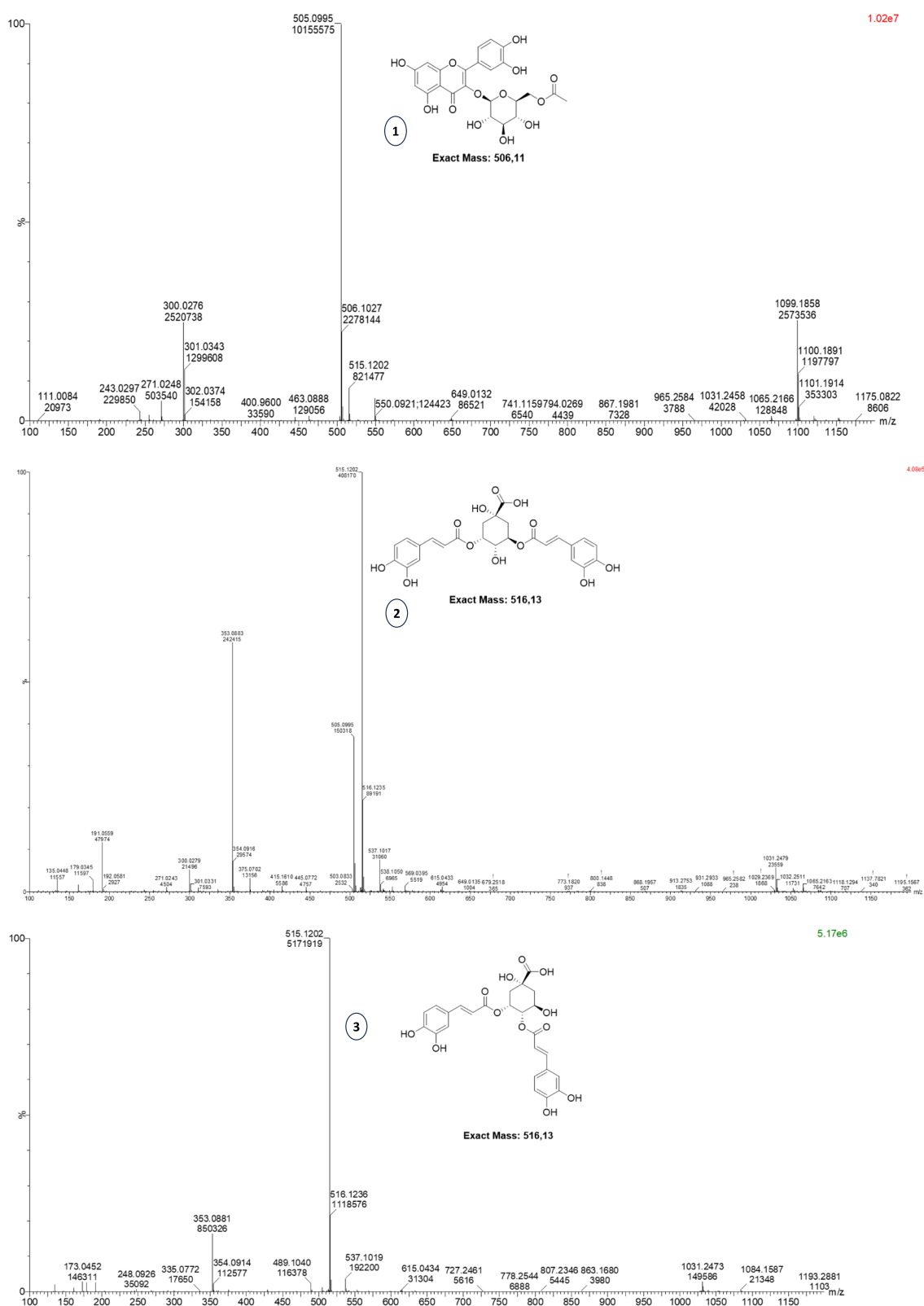


Fig. 5 Mass spectra of the major chemical compounds from the most active fraction EF-20: 1 = Quercetin 3-O-(6'-acetyl-glucoside); 2 = 3,5-Dicafeoylquinic acid; 3 = 3,4-Dicafeoylquinic acid

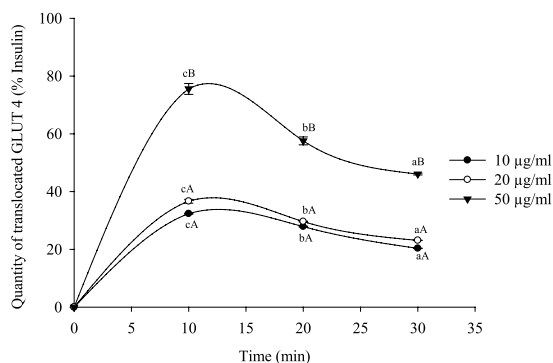
50 $\mu\text{g/mL}$ (and also 20 $\mu\text{g/mL}$) of 3,4-dicaffeoylquinic acid increases the amount of translocated GLUT4 to $36.05 \pm 0.96\%$ Insulin after only 10 min of activity (Fig. 6B). Likewise, as time increases, the activity of 3,5-dicaffeoylquinic acid on GLUT4 translocation, expressed as a percentage of insulin, tends to improve (Fig. 6C). Maximum amounts of translocated GLUT4 are observed after just 10 min of activity, notably $116.00 \pm 3.00\%$ Insulin for a concentration of 50 $\mu\text{g/mL}$ of 3,5-dicaffeoylquinic acid.

It is noteworthy that among the three bioactive compounds with known chemical structures, 3,5-dicaffeoylquinic acid at 50 $\mu\text{g/mL}$ exhibited the highest GLUT4 translocation activity of $116.00 \pm 3.00\%$ Insulin after 10 min. In other words, this means that after 10 min, 3,5-dicaffeoylquinic acid at 50 $\mu\text{g/mL}$ increases the amount of translocated GLUT4 by 1.16 times compared to insulin. Compared to various rapid-acting insulins (15 min post-injection) used to control postprandial blood glucose levels (Lispro, aspart, glulisine) [25] in diabetic patients, 3,5-dicaffeoylquinic acid could therefore

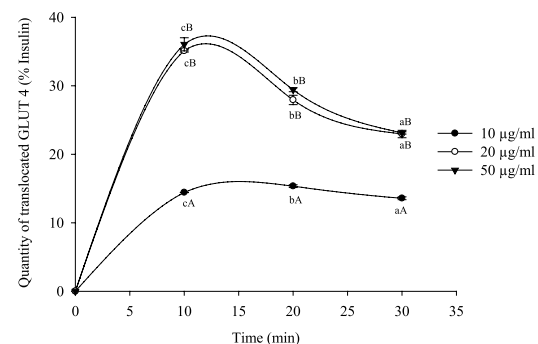
exhibit rapid action in regulating blood glucose. However, it could not be suitable for regulating blood glucose between meals and at night (action lasting 1 to 3 h) [25, 26]. Very few authors have reported on the effects of 3,5-dicaffeoylquinic acid concentration on GLUT4 translocation activity. The same observation is noted with 3,4-dicaffeoylquinic acid, in contrast to quercetin 3-O-(6"-acetyl-glucoside), which is known for its ability to enhance GLUT4 translocation [27, 28]. Most studies focus on antioxidant activities [29], anti-inflammatory effects [30], protective effects [31, 32], etc., with very few addressing GLUT4 translocation. As 3,5-dicaffeoylquinic acid was most active compound, it was isolated and suggested to 1D and 2D NMR analysis in order to clarify its structure. The NMR spectra are available in supplementary data.

Conclusion

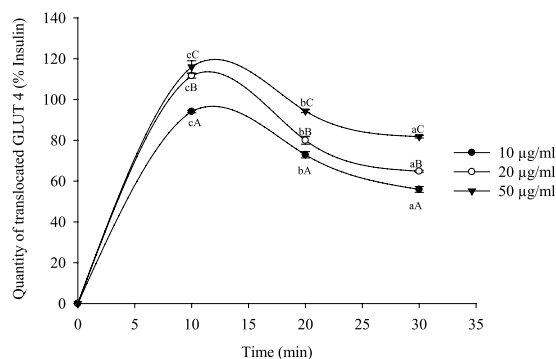
The extracts from the powdered leaves of *C. olitorius* are rich in several families of bioactive compounds, notably polyphenols, flavonoids, organic acids, and a peptide hormone. However, not all these families of compounds



A: Quercetin 3-O-(6"-acetyl-glucoside) activity: Time: Sum of squares = 19.47%, $P = 0.0001$; Compound concentration: Sum of squares = 76.45%, $P = 0.0001$; Residual: Sum of squares = 4.06%



B: 3,4-Dicaffeoylquinic acid activity: Time: Sum of squares = 18.94%, $P = 0.0001$; Compound concentration: Sum of squares = 72.74%, $P = 0.0001$; Residual: Sum of squares = 8.30%



C: 3,5-Dicaffeoylquinic acid activity: Time: Sum of squares = 73.64%, $P = 0.0001$; Compound concentration: Sum of squares = 24.30%, $P = 0.0001$; Residual: Sum of squares = 2.04%

Fig. 6 GLUT4 translocation activities of the three compounds (Quercetin 3-O-(6"-acetyl-glucoside); 3,5-Dicaffeoylquinic acid; 3,4-Dicaffeoylquinic acid) at different concentrations (10, 20, 50 $\mu\text{g/mL}$) on GLUT4 translocation over time (means marked with different lowercase letters (time effect) and uppercase letters (concentration effect) indicate significant differences at a probability threshold of $P < 0.05$)

exhibit GLUT4 translocation activity. Only those belonging to the flavonoid family and quinic derivatives, including quercetin 3-O-(6"-acetyl-glucoside)), 3,5-dicaffeoylquinic acid and 3,4-dicaffeoylquinic acid, increase the amount of translocated GLUT4. These increases in GLUT4 levels depend on the extract concentration and the duration of the activity. Quercetin 3-O-(6"-acetyl-glucoside) and 3,4-dicaffeoylquinic acid are strongly influenced by the extract concentration, while 3,5-dicaffeoylquinic acid is primarily influenced by time. Nonetheless, 3,5-dicaffeoylquinic acid exhibits the highest GLUT4 translocation activity (1.16 times greater than that of insulin) after 10 min at a concentration of 50 µg/ml and may contribute to a rapid reduction in postprandial blood glucose levels in diabetic patients. Consequently, these findings further support the consumption of powders from this leafy vegetable, commonly consumed as a dish, as a functional food (in the form of dietary supplements) for these patients. However, the mechanism of action of 3,5-dicaffeoylquinic acid on GLUT4 translocation will be the subject of a future study.

Supplementary Information

The online version contains supplementary material available at <https://doi.org/10.1007/s44403-025-00030-3>.

Supplementary Material 1.

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Authors' contributions

AAJA: Experimentation, Methodology, Data Collection, Data Analysis and Processing, Design, Writing and correction of the original draft; AODS: Methodology, Writing and correction of the original draft; NE: Methodology, Correction of the original draft, Supervision.

Funding

Not applicable.

Data availability

The data sets generated and analyzed during the current study are available from the corresponding author upon reasonable request.

Declarations

Ethics approval and consent to participate

Not applicable.

Competing interests

The authors declare that they have no conflict of interest.

Author details

¹Department of Home Economics, Advanced Teacher's Training College for Technical Education, University of Douala, 24 157 Douala, Cameroon.

²Department of Biological Sciences, Faculty of Medicine and Pharmaceutical Sciences, University of Douala, 2701 Douala, Cameroon. ³Department of Food Sciences and Nutrition, National High School of Agro-Industrial Sciences, University of Ngaoundere, 455 Ngaoundere, Cameroon.

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