Costus afer Ker Gawl (Bush cane) extracts modulate glucose uptake, triglyceride accumulation and oxidative stress in human SW 872 liposarcoma and HepG2 hepatocarcinoma cells

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Abstract

The current study investigates the biological effects of *Costus afer* (CAL and CAS extracts), a plant with known antidiabetic properties used in traditional medicine in West and tropical Africa, on pathways involved in type 2 diabetes mellitus (T2DM) in human adipocyte (SW 872) and hepatocyte (HepG2) cell models.

The cytotoxicity of CAL and CAS extracts was assessed using the MTS assay, while their influence on glucose uptake in HepG2 and SW 872 cells and triglyceride accumulation in oleic acid-differentiated SW 872 cells were studied. The study also examined the in vitro antioxidant activity (expressed in Trolox equivalents), the production of reactive oxygen species (ROS) induced by H_2O_2, and the anti-inflammatory effects, as demonstrated by the inhibition of albumin denaturation.

The extracts demonstrated no toxicity at concentrations between 1–50 µg/mL and significantly promoted glucose uptake in SW 872 cells (+ 46.7% and + 69.0%) and HepG2 cells in a dose-dependent manner (+ 42.6% and + 45.3%). Furthermore, CAL and CAS reduced triglyceride accumulation in differentiated SW 872 cells (CAL: − 34.6%; CAS: -38.4%) and displayed strong antioxidant activity, particularly CAS (11.38 ± 0.7 µM Trolox equivalent/g). Both extracts also reduced reactive oxygen species (ROS) production at 20 µg/mL and exhibited notable anti-inflammatory effects, inhibiting albumin denaturation by over 70% at 50 µg/mL and over 90% at 100 µg/mL.

Costus afer presents significant therapeutic potential for managing type 2 diabetes and obesity. This research underscores the plant's promise as a natural treatment option for addressing metabolic disorders.

Highlights

Costus afer Ker Gawl is a medicinal plant traditionally used in Cameroon to treat diabetes mellitus

Costus afer extracts enhanced glucose uptake in SW 872 and HepG2 cells, with the highest effects at 50 µg/mL

Costus afer extracts exhibited a highest antioxidant potential, and significantly reduced lipid accumulation and reactive oxygen species production in SW 872 and HepG2 cells.

The findings suggest that *Cotus afer* extracts are promising treatment option for managing Type 2 diabetes mellitus (T2DM) and related conditions such as obesity and cardiovascular disease.

1. Introduction

Type 2 diabetes mellitus (T2DM) is a multifactorial disorder, characterized by fasting and postprandial hyperglycemia, mainly caused by insulin resistance, consisting in the failure of normal insulin levels to stimulate glucose uptake (Galicia-Garcia et al., 2020) by insulin-sensitive tissues, such as liver, adipose, and skeletal muscle (Roden and Shulman, 2019), in combination with a polygenic background. A high-calorie diet, rich in fats and carbohydrates, raises blood glucose levels and increases circulating triglyceride-rich lipoproteins such as very-low-density lipoproteins (VLDLs), chylomicrons (CMs), and their remnants (CMRs). This elevation leads to heightened production of reactive oxygen species (ROS), which subsequently promotes the excessive formation of pro-inflammatory mediators (Dali-Youcef et al., 2013). Unbalanced nutrition, along with additional environmental factors (sedentarism, obesity, age) associated to the development of T2DM, triggers a pro-inflammatory response leading to insulin resistance and endothelial dysfunction (Guarner and Rubio-Ruiz, 2015). Moreover, an impaired response to insulin stimulation by the adipose tissue will lead to an impaired suppression of lipolysis and glucose uptake, and an enhancement of free fatty acids (FFA) release into the circulation even in the presence of high insulin levels (Czech, 2020). As the pathophysiology of T2DM and the underlying mechanisms are increasingly understood, precision medicine should be implemented with appropriate individualized and targeted treatments (Usova et al., 2021). Moreover, appropriate experimental models may help to both clarify such mechanisms and to identify potentially useful nutraceutical compounds to prevent this condition. HepG2 cells, commonly used as a model for human hepatocytes, have been employed extensively in studies investigating glucose uptake (Atchan Nwakiban et al., 2021; Hu and Wang, 2011). In addition, the human SW 872 cell line has been used in previous studies as a human adipocyte cell model (Chiarelli and Di Marzio, 2008; Cicolari et al., 2020) and can be further differentiated to mature adipocytes by oleic acid treatment (Wassef et al., 2004). Fully differentiated adipocytes respond to insulin and absorb glucose more efficiently than preadipocytes, with greater GLUT4 translocation to the cell membrane by insulin, leading to increased glucose uptake at lower insulin levels (Kanzaki and Pessin, 2001).

Some drugs used for the treatment of T2DM lead to the development of obesity as a side effect by reducing blood glucose levels and inducing adipogenesis. The therapy may act by mimicking insulin or either stimulating insulin release or by potentiating insulin action or reducing hepatic glucose production. For example, insulin sensitizers like thiazolidinediones (TZDs), a class of oral antidiabetic agents, is used to improve insulin resistance mainly through the promotion of adipogenesis and reduction of free fatty acid influx into skeletal muscle and liver (Atchan Nwakiban et al., 2021; Cicolari et al., 2020; Nwakiban et al., 2020a). However, their adverse effects limit their long-term use. Hence, the demand for new anti-diabetic or anti-obesity compounds continues (Chiarelli and Di Marzio, 2008). Traditional herbal and/or nutritional remedies can be used in the treatment of pre-diabetes and T2DM by acting on adipocytes and can act as a better alternative for the treatment of metabolic disorders (Payab et al., 2020). The use of natural medicines and their phytochemical compounds for T2DM management is not only a priority for developing safer alternatives to pharmaceutical products, which transitorily lower blood glucose and prevent high blood pressure, but also to enhance antioxidant defenses and insulin action and secretion. Therefore, it is important to identify and validate compounds capable to modulate some intracellular pathways implicated in T2DM pathophysiology, especially if they are already used in ethnomedicine, but the evidence of their antidiabetic activity is often anecdotal (Chang et al., 2013; Kumar et al., 2021). Amongst other plants, *C. afer,* of the Costaceae family, is largely used in traditional medicines to manage diabetes and other diseases. Known as monkey sugar cane or bush sugar cane, population regularly

consumed its juice from the stem, which is sour in taste, to cure coughs. This plant is commonly found in the moist and shady forests of West and tropical Africa, it is a tropical monocot plant and relatively herbaceous, tall, with no branches and creeping rhizome. *C. afer,* is a perennial, rhizomatous plant that may reach a height of up to 4 m. The leaves are simple and spirally laid out. The sheath is a closed, tubular structure with green coloring and distinctive purple blotches. The ligule, which measures between 4 and 8 mm, is leathery and smooth. Petioles range from 4 to 12 mm in length. The leaf blade has an obovate elliptic shape, typically measuring 15–35 cm in length and 3.5–9.5 cm in width. Its base is rounded to subcordate, while the apex is acuminate. The margin is sparsely hairy, generally smooth on the upper surface, though sometimes slightly hairy beneath. The flowers are bisexual and exhibit zygomorphism (Boison et al., 2019; Tchamgoue et al., 2015). Analysis of different leaves and stems of *C. afer* indicates the presence of fat, ash, carbohydrate, crude proteins, and fibers. Some vital nutrients such as vitamins B, E and C are also reported to be present in the leaves. Characterization of the fatty acid profile of *C.afer*-derived oil shows predominance of saturated (78%) and unsaturated (22%) fatty acids, featuring key compounds such as abinene, β-pinene, and β-caryophyllene (Boison et al., 2019; Ekpe et al., 2018). Our previous studies on crude solvent extracts of *C. afer* revealed its antihyperglycemic activity through several mechanisms: (i) inhibition of the carbohydrate hydrolyzing enzymes (Tchamgoue et al., 2015), (ii) inhibition of glucose uptake by yeasts cells (Tchamgoue et al., 2016) and, (iii) capability to regenerate β-pancreatic cells and to possess *in vitro* antioxidant properties (Tchamgoue et al., 2018). The chemical investigations of *C. afer* using HPLC fingerprinting reveals a rich spectrum of bioactive metabolites such as flavonoids (kaempferol-3-O- α-L-rhamnopyranoside), phenols, cardiac glycosides, anthraquinones, saponins, terpenoids, alkaloids and tannins (Anyasor et al., 2014; Boison et al., 2019; Tchamgoue et al., 2015). However, no study has investigated the biological activity of *C. afer* at the cellular level.

In this regard, this study aimed to investigate the effects of *C. afer* leaves (CAL) and *C. afer* stems (CAS) extracts in the human SW 872 liposarcoma and HepG2 hepatocarcinoma cells lines on molecular pathways related to T2DM pathophysiology, such as glucose uptake, triglyceride accumulation and antioxidant activity.

2. Materials and Methods

2.1. Materials

Bovine serum albumin (BSA), 2'-Azobis (2-methylpropionamidine) dihydrochloride (AAPH), dimethyl sulfoxide (DMSO), bovine insulin, hydrogen peroxide (H_2O_2), (±)-6-hydroxy 2,5,7,8 tetramethylchromane-2-carboxylic acid (Trolox), oleic acid, resveratrol and metformin were obtained from Sigma-Aldrich Co. (Saint-Louis, MO, USA). The compound 2-deoxy-2-[(7-nitro-1,2,3-benzoxadiazol-4-yl)amino]-D-glucose (2-NBDG) was sourced from Abcam (Cambridge, MA, USA), while 5-(and-6)-chloromethyl-2',7'-dichlorodihydrofluorescein diacetate (CM-H2DCFDA) was procured from Thermo Fisher Scientific (Rodano (MI), Italy).

2.2. Preparation of plant extracts

C. afer plant was harvested from their natural habitat in Yaoundé (Cameroon) with the assistance of an ethnobotanist. Selected samples were made up of leaves and stems (Fig. 1) identified in the National Herbarium of Cameroon in Yaoundé (Cameroon) based on a comparison with the preserved specimens (Table 1). The preparation of the methanolic extracts was conducted as previously reported (Tchamgoue et al., 2018). Air-dried and powdered samples of leaves and stems (each weighing 1400 g) were subjected to extraction with 5 liters of 100% methanol at room temperature, protected from light, for a duration of 72 hours. The mixture was filtered, concentrated under reduced pressure, frozen to produce crude extracts, and dried in an oven (50°C) for 3 days. The extracts stock solutions (100 µg/mL) were dissolved in DMSO and aliquoted, then kept at − 80°C for further experiments. The stock solution of DMSO-solubilized extracts was diluted in a culture medium at concentrations appropriate for cellular treatment, with a final concentration of DMSO never greater than 0.1%.

Table 1
Identification of *C. afer* extracts

Plant name	Part used	Family	Herbarium voucher number	Extract color	Extract aspect	Extraction yield (%)
Cotus afer Ker Gawl	Leaves	Costaceae	NHC 11708	Dark brown	Pasty	36.3
	Stems			Beige brown	Pasty	27.4

2.3. Cell cultures and differentiation of adipocytes

The human hepatocellular carcinoma cell line (HepG2, ATCC® HB-8065TM) and the liposarcoma cell line (SW-872, ATCC® HTB-92TM) were obtained from the American Type Culture Collection (ATCC®, Manassas, VA, USA) and cultured according to the provider's guidelines. They were respectively cultured in MEM (Minimum Essential Medium Eagle) and DMEM-F12 culture media (Dulbecco's Modified Eagle Medium: Nutrient Mixture F-12) containing 15 mM of HEPES buffer (2- (4- (2-Hydroyethyl)-1 -piperazinyl)-ethansulfonsaure. The media were enriched with 10% fetal bovine serum (FBS), along with 1% penicillin (100 U/mL) and streptomycin (100 µg/mL). Upon reaching 80–90% confluence, SW 872 cells were exposed to 100 µM oleic acid (OA) for 7 days to promote differentiation into adipocytes (Atchan Nwakiban et al., 2021).

2.4. Cytotoxicity assay and cell treatment

The cytotoxic effects of the plant extracts were evaluated by determining cell viability using the MTS assay [3-(4,5-Dimethylthiazol-2-yl)-5-(3-Carboxymethoxyphenyl)-2-(4-Sulfophenyl)-2H-tetrazolium]. Cells were exposed to varying extract concentrations (0–50 µg/mL) for 24 hours. This was carried out using the Cell Titer 96 aqueous non-radioactive cell proliferation assay (Promega, Madison, WI, USA) as described by (Atchan Nwakiban et al., 2021). To evaluate cell viability, cells were seeded in sterile, flat-bottomed 96-well plates at a density of 2×10^4 cells per well and incubated for 24 hours at 37°C in a humidified atmosphere with 5% CO_2. Treatment solutions, prepared at varying concentrations (1, 10, 25, and 50 µg/mL) in serum-free MEM or DMEM-F12

media, were added (100 µL per well), followed by another 24-hour incubation. MTS reagent, combined with phenazine methosulfate as an electron coupling agent, was then introduced (20 µL per well) and incubated for 1 hour at 37°C. After gently shaking for 2 minutes, absorbance was measured at 490 nm using a multimode plate reader (EnSpire, PerkinElmer, NYC, USA). Controls and blanks, including DMSO-treated cells (0.1%) and wells with cell-free media, were incorporated, and cell viability was determined by measuring absorbance readings and applying the following calculation formula:

$$\varvec{\%}\ \varvec{c}\varvec{e}\varvec{l}\varvec{l}\ \varvec{v}\varvec{i}\varvec{a}\varvec{b}\varvec{i}\varvec{l}\varvec{i}\varvec{t}\varvec{y} = \frac{}{\var}$$

Three distinct experiments run in triplicate were conducted.

2.5. Morphological Analysis

Cells were cultured in sterile flat-bottom 6 cm² dishes at a density of 5 × 10^5 cells per dish, as described above. The cultures were incubated at 37°C in a humidified incubator with 5% CO_2 for 24 hours. Two concentrations of C. afer extracts (1 µg/mL and 50 µg/mL) were prepared in fresh serum-free media, and 3 mL of each treatment was added to the dishes, followed by another 24-hour incubation. After treatment, the cells were examined using a ZEISS microscope (ZEISS, VA, USA) at magnifications of 10× and 32×.

2.6. Lipid Content Measurement (Oil Red O method)

To assess the intracellular effect of leaves and stem *C. afer* extracts on lipid accumulation of SW 872 cells, we used the Oil Red O (ORO) staining method. SW 872 cells were plated in twenty-four-well plates and allowed to reach 90–100% confluence before being treated with 100 µM oleic acid (OA) for a duration of 7 days, following the methodology outlined by (Atchan Nwakiban et al., 2021). Following differentiation, the SW 872 cells were subjected to treatments with varying concentrations of leaf and stem extracts of *C.afer* for 24 hours. After removing the culture medium, saline phosphate buffer (PBS) was used to wash the cells and then they were fixed with 4% formaldehyde in PBS at room temperature for 1 hour. The lipid accumulation was assessed with the addition of the working solution (0.2% ORO in 40% isopropanol) to the culture plates and kept incubated for 20 minutes at room temperature. The ORO stain was then eluted using 100% isopropanol, which was added to the plates to quantify the lipid content. The plates were gently shaken on an orbital shaker for 10 minutes at room temperature, and 200 µl of the eluate was transferred to a clear polystyrene 96-well microtiter plate for analysis. Absorption was measured in duplicate at 520 nm using a microplate reader (EnSpire PerkinElmer) with a dynamic range of 0.000 O.D. to 0.200 O.D. Three distinct experiments run in triplicate were conducted.

2.7. Glucose uptake assay

Glucose uptake was assessed by quantifying the absorption of 2-desoxy-2-[(7-nitro-2, 1, 3 benzoxadiazol-4-yl) amino]-D glucose (2-NBDG) as described by (Nwakiban et al., 2020a). Briefly, cells were cultured in 96-well black plates for 48 h in DMEM high glucose to induce insulin resistance. Different concentrations of extracts (10, 20, 50 µg/mL) or 10 µM metformin were added and incubated for 24 h without FBS. Subsequently, to determine the absorption of 2-NBDG, cells were incubated with 80 µM 2-NBDG and 0.1 nM insulin (dissolved in a glucose-free medium) for 30 minutes and then washed two times with ice-cold PBS to stop further uptake. The fluorescence intensity of 2-NBDG was quantified using a microplate reader (EnSpire, PerkinElmer, NYC, USA), with excitation and emission wavelengths set at 465 nm and 540 nm, respectively. To determine the rate of glucose uptake, the following calculation was employed:

$$\varvec{\%}\ \varvec{G}\varvec{l}\varvec{u}\varvec{c}\varvec{o}\varvec{s}\varvec{e}\ \varvec{u}\varvec{p}\varvec{t}\varvec{a}\varvec{k}\varvec{e}\ \varvec{i}$$

Three distinct experiments run in triplicate were conducted and FI is the fluorescent signal.

2.8 Measurement of radical oxygen species production

The level of intracellular radical oxygen species (ROS) in the cells was determined by a fluorometric test, using the 5-(and-6)-chloromethyl-2',7'-dichlorodihydrofluorescein diacetate (CM-H2DCFDA) oxidant-sensitive fluorescence probe, according to the method described by (Nwakiban et al., 2020b). Cells were cultured in black 96-well plates and allowed to reach approximately 90% confluence before being treated with extracts from *C. afer* at different concentrations (0–50 µg/mL) for a duration of 24 hours. Following this, the cells were treated with 20 µM CM-H2DCFDA and subsequently exposed to 500 µM hydrogen peroxide for one hour to induce the production of reactive oxygen species (ROS). The resulting fluorescence intensities were recorded using the EnSpire PerkinElmer multimode plate reader, with excitation and emission wavelengths established at 485 nm and 535 nm, respectively. Three distinct experiments run in triplicate were conducted.

2.9. In vitro anti-inflammatory activity

C. afer extracts were screened for *in vitro* anti-inflammatory activity by using inhibition of albumin denaturation technique as earlier described by (Gondkar et al., 2013) with some modifications. Test solution (1 ml) containing different concentrations (50 and 100 µg/mL) of extracts and the standard drug (diclofenac sodium) was mixed with 450 µL of bovine serum albumin (BSA) (5% w/v aqueous solution) and 1.4 mL of phosphate buffered saline (PBS). Distilled water instead of extracts with the above mixture was used as a negative control. Following the incubation of the mixtures at 37°C for 15 minutes, they were subsequently heated to 70°C for 10 minutes. The turbidity was then assessed at a wavelength of 660 nm after allowing the samples to cool. The experiment was carried out in triplicates and the percentage of inhibition of denaturation was calculated using the following equation and from control where no drug was added:

$$\varvec{\%}\ \varvec{I}\varvec{n}\varvec{h}\varvec{i}\varvec{b}\varvec{i}\varvec{t}\varvec{i}\varvec{o}\varvec{n}\ \varvec{o}\varvec{f}\ \varvec{d}\varvec{e}$$

2.10. Oxygen Radical Absorbance Capacity (ORAC) Assay

The oxygen radical absorption capacity (ORAC) assay was performed using the method described above (Ou et al., 2001) with minor modifications. Briefly, 20 µL of each extract stock solution (1 µg/mL) was distributed into a black 96-well plate. To each well, 120 µL of fluorescein solution, prepared to a final concentration of 70 nM in a phosphate buffer (pH 7.4, 75 mM), was added. Peroxyl radicals were generated by introducing 60 µL of AAPH at a concentration of 40 mM (Sigma-Aldrich, St. Louis, MO, USA). The concentration of each extract in the wells was subsequently standardized to 0.1 µg/mL. Fluorescence measurements were subsequently recorded using a spectrophotometer (Victor X3, PerkinElmer, USA), with excitation and emission wavelengths set to 484 nm and 528 nm, respectively.After shaking, the fluorescence was recorded every 2 minutes for a total duration of 60 minutes at 37°C. Trolox, with concentrations ranging from 0 to 50 µM, served as a reference inhibitor. The area under the curve (AUC) for each extract was determined, and the results were reported as µM Trolox equivalent. This procedure was replicated across three independent experiments, each conducted in triplicate.

2.11. Statistical analysis

The results from a minimum of three independent experiments, each conducted in triplicate, are presented as mean ± standard deviation (SD) values or as a percentage (%) relative to a control group. Statistical analysis was performed using a one-way analysis of variance (ANOVA), followed by multiple comparison assessments via the Bonferroni post hoc test. All statistical computations and graphical representations were generated using GraphPad Prism 9.0 software (GraphPad Software Inc., San Diego, USA).

3. Results

3.1. Effects of C.afer extracts on cell viability and morphology

The cytotoxicity of *C.afer* extracts was assessed at concentrations of 1–50 µg/mL, in both human SW 872 and HepG2 cells by the MTS assay. The viability threshold was set at 80%. After 24 h treatment, no cytotoxic effects were observed for both cell lines (Fig. 2).
We further explored whether the extracts influenced cell morphology alongside cell viability. After 24 hours of treatment, no noticeable changes in cell morphology were observed (Figs. 3 and 4). Based on these results, all treatments with extracts were carried out for 24 h at concentrations of 50 µg/mL or below.

3.2. Effects of C.afer extracts on glucose uptake

In the presence of insulin, glucose uptake significantly increased in both HepG2 (+ 30.8; $p < 0.01$) and SW 872 (+ 53.3%; $p < 0.001$) cells (Fig. 5) compared to untreated (basal glucose uptake) cells. Glucose uptake was significantly more pronounced in cells treated with 10 µM metformin compared to untreated cells (+ 51.9%; $p < 0.001$ in HepG2 and + 46.7%; $p < 0.001$ in SW 872) and cells treated with insulin (+ 21.2%; $p < 0.01$ in HepG2 and + 18.6%; $p < 0.05$ in SW 872). The effect of CAL and CAS extracts was tested at the concentrations of 10, 20 and 50 µg/mL. In HepG2 cells, CAL showed a concentration-dependent stimulation of glucose uptake (+ 12.6% at 20 µg/mL; $p < 0.05$, + 42.6% at 50 µg/mL; $p < 0.01$) (Fig. 5A). In these cells, CAS also showed a concentration-dependent stimulation of glucose uptake (+ 11.4% at 20 µg/mL; $p < 0.05$, + 45.4% at 50 µg/mL; $p < 0.01$) (Fig. 5B). In SW 872 cells, exposure to CAL resulted in a significant reduction of glucose uptake at the lower concentrations (-28.4% at 10 µg/mL; $p < 0.01$, -16.8% at 20 µg/mL; $p < 0.05$), while at 50 µg/mL glucose uptake was strongly stimulated (+ 46.7%; $p < 0.001$) (Fig. 5A). Similarly, CAS also significantly reduced glucose uptake at the lowest concentration (-22.9% at 10 µg/mL; $p < 0.05$), its effect was neutral at 20 µg/mL, while at 50 µg/mL glucose uptake was again strongly stimulated (+ 69.1%; $p < 0.001$) (Fig. 5B).

3.3. Effect of C. afer extracts on triglyceride accumulation in differentiated adipocytes

Differentiation of SW872 cells with oleic acid (OA, 100 µM) resulted in a significant ($p < 0.01$) increase (+ 27.5%) in triglyceride accumulation, compared to untreated cells (Fig. 6). After a 24-h exposure of differentiated SW 872 cells to both CAL and CAS extracts at 1, 10 and 20 µg/mL, we observed a concentration-dependent decrease of triglyceride content, which reached significance at the maximum concentration tested (20 µg/mL; CAL: -34.6%; $p < 0.01$, CAS – 38.4%; $p < 0.01$) in comparison with untreated differentiated SW 872 cells. Treatment of differentiated SW 872 cells with resveratrol (10 µM) and metformin (10 µM), taken as active controls, shows a higher activity with a significant ($p < 0.01$) reduction (-49.2% and – 35.7% respectively) of the triglyceride content.

3.4. Effect of extracts on intracellular ROS production.

One-h treatment with H_2O_2 enhanced ROS production by approximatively 50% in both cell lines (Fig. 7). In HepG2 cells, CAL extract significantly ($p < 0.05$) enhanced ROS production (+ 11.7%) at 10 µg/mL, was neutral at 20 µg/mL and significantly reduced ROS production at 50 µg/mL (-44.2%; $p < 0.01$) (Fig. 7A). Interestingly, CAS extract was significantly effective in reducing H_2O_2-induced ROS production at all tested concentrations (range – 59.7/-66.3%) compared to H_2O_2-treated cells (Fig. 7B). Concerning SW 872 cells, CAL significantly reduced ROS production at 10 µg/mL (-17.4%, $p < 0.05$), at 20 µg/mL (-31.8%, $p < 0.01$) and at 50 µg/mL (-50.1%, $p < 0.001$) (Fig. 7A). Treatment with CAS significantly resulted in increased ROS production at 10 µg/mL (+ 22.8%, $p < 0.05$), but reduced it at 20 µg/mL (-34.6%, $p < 0.01$) and at 50 µg/mL (-49.4%, $p < 0.001$) (Fig. 7B).

3.5. Effect of C. afer extracts on protein denaturation and peroxyl radical cell-free system production

The *C. afer* extracts were tested for *in vitro* anti-inflammatory activity by using inhibition of albumin denaturation technique compared to standard diclofenac (Fig. 8). At a concentration of 50 µg/mL, both CAL and CAS extracts exhibited notable in vitro anti-inflammatory effects, as indicated by an albumin denaturation inhibition rate exceeding 70%. This effect became even more pronounced at 100 µg/mL, where inhibition rates surpassed 90%. However, this

activity was less pronounced than the standard drug (diclofenac sodium) at 100 µg/mL (Fig. 8A). The ORAC results were expressed as Trolox equivalent (TE). Net areas under the fluorescein decay curve (AUC) with increasing dosage of Trolox demonstrated a linearity correlation with R^2 value 0.9875. At 0.1 µg/mL, leaves and stem extracts of *C. afer* showed significant ($p < 0.05$) activity with a value of 5.9 ± 1.6; 11.38 ± 0.7 µM Trolox equivalent g of extract respectively (Fig. 8B).

4. Discussion

Costus afer Ker Gawl, from *Costaceae* family, known as bush sugar cane, is commonly found in West and tropical Africa. *C. afer* is used by the local folks for the treatment and management of diseases such as inflammation, T2DM, and hepatic disorders (Boison et al., 2019). In our previous studies, we showed, using different approaches, that *C. afer* possesses the capability to inhibit carbohydrates hydrolyzing enzymes, to inhibit glucose uptake by yeasts cells, to regenerate β-pancreatic cells, and to possess *in vivo* hypoglycemic and antioxidant activities (Tchamgoue et al., 2016, 2020a, 2018, 2015). In these previous works, the extracts of different parts (leaves, stem and rhizomes) of *costus afer* had been obtained following a successive extraction with different solvents (Hexane, ethyl acetate, methanol and water). The methanolic extracts of leave and stem showed the best activity, which justifies the choice of these extracts during this study. Based on those experiments, and although the present study has the limits of being carried out i*n vitro* on cells, we studied the mechanism of action of extracts from leaves and stem of *C. afer* through their effects on cytotoxicity, glucose uptake, intracellular triglyceride accumulation, antioxidant and anti-inflammatory activities. Preliminary experiments were carried out to evaluate the effect of *C. afer* extracts on the viability of HepG2 and SW 872 cells by an MTS assay and morphological analysis. Similar to our earlier study (Atchan Nwakiban et al., 2021; Nwakiban et al., 2020a), no cytotoxic effects of extracts were observed in the cell cultures when treated with extracts. Therefore, they were used for further experiments. *C. afer* extracts at 50 µg/mL were shown to increase glucose uptake in HepG2 hepatoma and differentiated SW 872 liposarcoma cell lines. In agreement with these findings, a similar trend was also observed with other plant species, including *Annona stenophylla*, which has been found to stimulate glucose transport in C2C12 myotubes with an increase in the total quantity of GLUT4 or its gene expression (Taderera et al., 2019). The extracts seem to influence protein trafficking in a targeted manner, indicating a potential enhancement of glucose uptake through the facilitation of molecular processes such as the translocation of glucose transporters to the cell membrane (Atchan Nwakiban et al., 2021; Nwakiban et al., 2020a, b).

In our study, we used the leaf and stem extracts of *C. afer*, in order to assess whether they can induce adipogenesis in the presence or absence of insulin. Metformin, a biguanide drug, was taken as positive control which is an insulin-sensitizing agent that acts by improving the sensitivity of peripheral tissues to insulin. The results showed that the *C. afer* leaf and stem extract, showed effects in a dose dependent manner similar to insulin sensitizing activities. The best activity was observed at concentration of 20 µg/mL for both extracts and the *C. afer* stem (CAS) extract performed (85.02%) almost like metformin (88.73%).

The sustained impairment of glucose uptake, along with insulin resistance and metabolic dysfunction, seems to be driven by the secretion of pro-inflammatory cytokines from dysfunctional cells, such as adipocytes, and the associated production of reactive oxygen species (ROS) (Atchan et al., 2023; Issa et al., 2018; Manna and Jain, 2015). On the basis of these considerations, we assessed the antioxidant, anti-inflammatory as well as intracellular inhibitory lipid accumulation activities of *C. afer* extracts in cells and cell-free systems. The extracts reduced the level of intracellular triglyceride storage in a concentration-dependent manner. Also, the findings demonstrated that *C. afer* extracts significantly reduced ROS production, effectively scavenged peroxyl radicals in a cell-free system (ORAC assay), and inhibited protein denaturation, a process linked to the development of inflammatory conditions. This effect could be attributed to compounds that help neutralize the excessive levels of reactive oxygen species (ROS), which arise from an imbalance between ROS production and the body's antioxidant defense systems. These compounds act as scavengers and are crucial in mitigating diabetic complications, insulin resistance, dyslipidemia, and cellular dysfunction (Atchan Nwakiban et al., 2021; Nwakiban et al., 2020a). n line with these findings, previous research has similarly demonstrated that extracts containing elevated levels of phenolic compounds exhibit enhanced biological activities across various domains (Ayalew et al., 2022; Olivares-Vicente et al., n.d.; Pace and Martinelli, 2022).

To date, no studies have investigated the biological properties of *C. afer* extracts (leaves and stem) in HepG2 and SW872 cell lines. In the present study, some mechanisms of action such as glucose uptake stimulation, triglyceride reduction, antioxidant and anti-inflammation were observed and the presence of certain bioactive compounds (flavonoids, phenolic acids, saponins, tannins, terpenoids and alkaloids) previously identified in this plant could be justified his biological activity. All these properties and some others have previously been demonstrated (Tchamgoue et al., 2016, 2020b, 2015), suggesting that *C. afer* could be a good candidate for the development of improved nutraceuticals and herbal medicine products for cardiometabolic disorders. This is more encouraging as its extracts have not exhibited cytotoxic effects in cell lines.

5. Conclusions

The present findings indicate that *C. afer* extracts possess a potential beneficial effect on cellular pathways relevant for T2DM and obesity onset and progression. Indeed, stem and leaf *C. afer* extracts show, at the highest concentration used in this study, hypoglycemic properties, antioxidant capacities, and inhibitory activity on triglyceride accumulation. On the contrary, less or opposite activities were observed at lowest concentrations. These findings raise important answers for further investigation of *C. afer* extracts, especially regarding the range of extract concentrations able to show on beneficial biological effects and the deduction of their molecular mechanism. This study demonstrated at the cellular level the safety and health value of *C. afer* extracts, which may be placed before drug treatment, thus being complementary to nutritional and pharmacological interventions.

Limitations: This study emphasizes the promising effects of *Costus afer* extracts (CAL and CAS) in managing type 2 diabetes mellitus (T2DM), demonstrated through *in vitro* assessments of glucose uptake, triglyceride accumulation, reduction of reactive oxygen species (ROS), and *in vitro* anti-inflammatory properties. However, the research has notable limitations, including the absence of *in vivo* models, a lack of elucidation regarding molecular mechanisms, a

narrow range of concentrations tested and a primary focus on individual plant extracts (no combination). To enhance therapeutic validation, future investigations should aim to address these deficiencies.

Abbreviations

AAPH: 2'-azobis (2-methylpropionamidine) dihydrochloride; ATCC: american type culture collection; ATP: Adenosine triphosphate ; AUC: area under the curve; CAL: *Cotus afer* leaf; CAS: *Cotus afer* stem; CM-H2DCFDA: 5-(and-6)-chloromethyl-2',7'-dichlorodihydrofluorescein diacetate; DNA: deoxyribo Nucleic Acid ; DMEM: (Dulbecco's Modified Eagle Medium; DMSO: dimethyl sulfoxide ; FBS: fetal bovine serum; FI: fluorescent signal; GLUT4: glucose transporter-4; HEPES: 2- (4- (2-Hydroyethyl)-1 -piperazinyl)-ethansulfonsaure H_2O_2: hydrogen peroxide; MEM: Minimum Essential Medium Eagle; MTS: 3-(4,5-Dimethylthiazol-2-yl)-5-(3-Carboxymethoxyphenyl)-2-(4-Sulfophenyl)-2H-tetrazolium; 2-NBDG: 2-deoxy-2-[(7-nitro-1,2,3-benzoxadiazol-4-yl) amino]-D-glucose; ORO: oil red O; OA: oleic acid; OD: optical density; ROS: reactive oxygen species; ORAC: oxygen radical absorbance capacity; T2DM: type 2 diabetes mellitus; TZDs: Thiazolidinediones; Trolox: (±)-6-Hydroxy 2,5,7,8 tetramethylchromane-2-carboxylic acid; SD: standard deviation.

Statements and Declarations

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Author Contributions: Armelle Deutou Tchamgoue and Achille Parfait Nwakiban Atchan contributed to the conceptualization, supervision, design, investigation, methodology and formal analysis of the study. Data curation, software and validation of results were performed by Achille Parfait Nwakiban Atchan. The first draft of the manuscript was written by Armelle Deutou Tchamgoue and Achille Parfait Nwakiban Atchan and all authors commented on previous versions of the manuscript.

Availability of data and materials: This manuscript has not been submitted or published elsewhere for publication. The data supporting this study can be found within the article.

Competing interests: The authors have no conflicts of interest to declare that are relevant to the content of this article

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Figures

Figure 1

Costus afer Ker Gawl (Bush cane) plant. Photos of the plant at the beginning (A) and end (B) of flowering and taken by Dr. Armelle Deutou Tchamgoue in Toussom, West, Cameroon on March 9, 2014

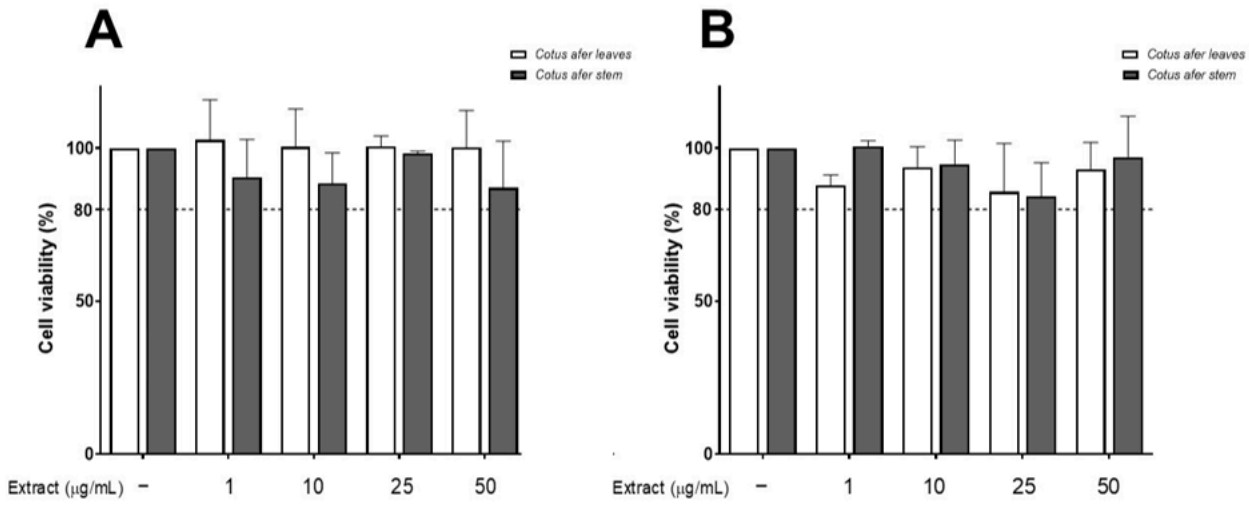

Figure 2

Effects of *C. afer* extracts on the viability of HepG2 (A) and SW 872 (B) cells, evaluated by the MTS assay. Cells were incubated for 24 h with different concentrations of plant extracts. Data are presented as a percentage of the control group, which is designated as 100; values are expressed as mean ± standard deviation (N=3). The threshold for cell viability is represented by the dotted line set at 80%. "–": no extract (control)

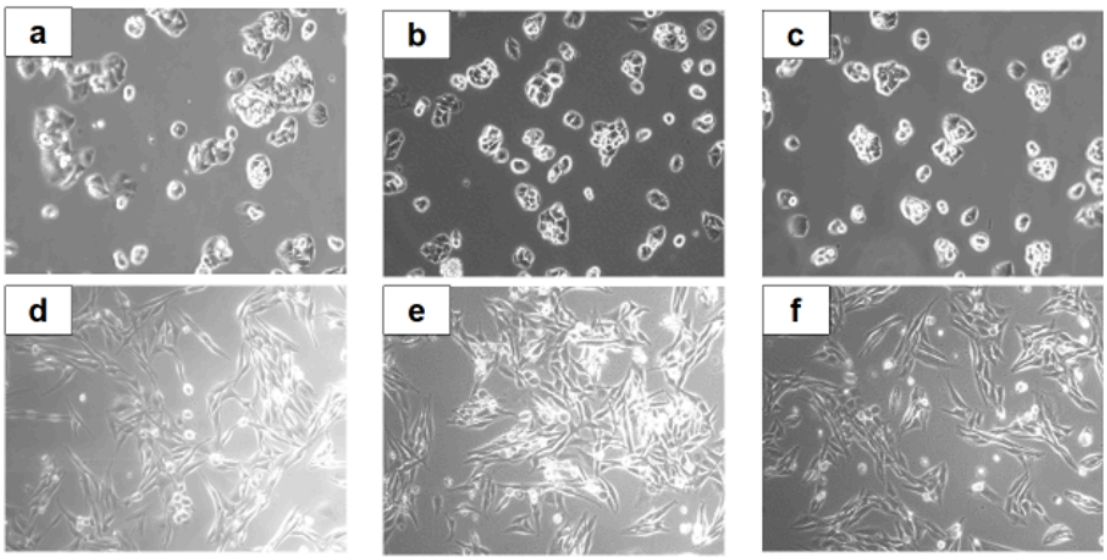

Figure 3

Morphological study of HepG2 and SW 872 cells after 24 h treatment with *C. afer* leaf extracts (inverted phase contrast microscopy; 10X magnification). The morphology of HepG2 (Fig. 3 a, b and c) and SW 872 (Fig. 3 d, e and f) cells was not affected by treatment. Extracts were tested at different concentrations: 0 µg/mL (Fig. 3 a), 1 µg/mL (Fig. 3 b) and 50 µg/mL (Fig. 3 c) in HepG2 and 0 µg/mL (Fig. 3 d), 1 µg/mL (Fig. 3 e) and 50 µg/mL (Fig. 3 f) in SW 872.

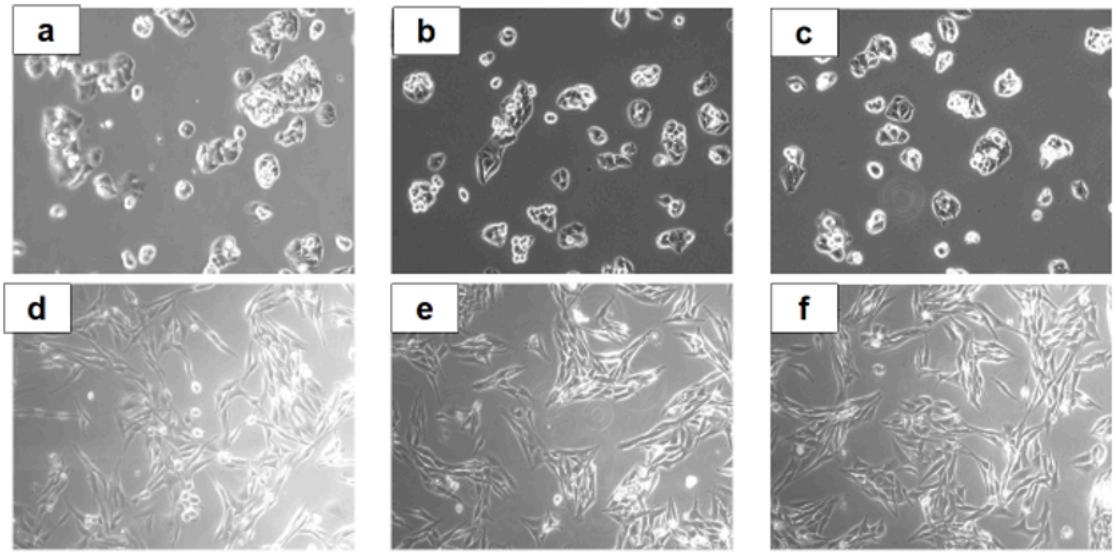

Figure 4

Morphological study of HepG2 and SW 872 cells after 24 h treatment with *C. afer* stem extracts (inverted phase contrast microscopy; 10X magnification). The morphology of HepG2 (Fig. 4 a, b and c) and SW 872 (Fig. 4 d, e and f) cells was not affected by treatment. Extracts were tested at different concentrations: 0 µg/mL (Fig. a), 1 µg/mL (Fig. 4 b) and 50 µg/mL (Figure 4 c) in HepG2 and 0 µg/mL (Fig. d), 1 µg/mL (Fig. e) and 50 µg/mL (Fig. f) in SW 872.

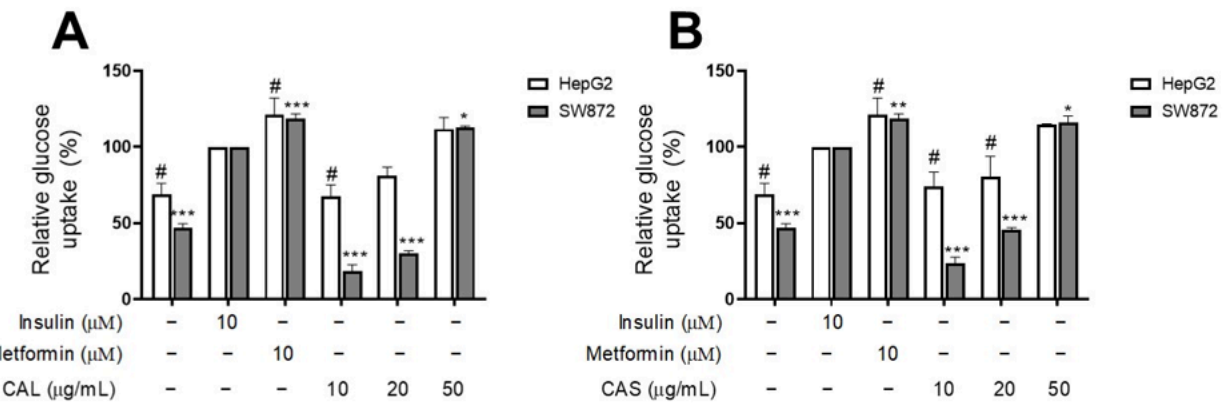

Figure 5

Effect of *C. afer*leaf (A) and stem (B) extracts on HepG2 and SW 872 cells glucose uptake. Cells were incubated for 24 h with extracts. Data are expressed as % of control (Insulin) taken as 100; mean SD, N=3. *p < 0.05, **p < 0.01, and ***p < 0.001 vs. SW 872 control group. #p < 0.05, ##p < 0.01 and ###p < 0.001 vs. HepG2 control group; CAL: *C. afer* leaves, CAS: *C. afer* stems. "–": Absent.

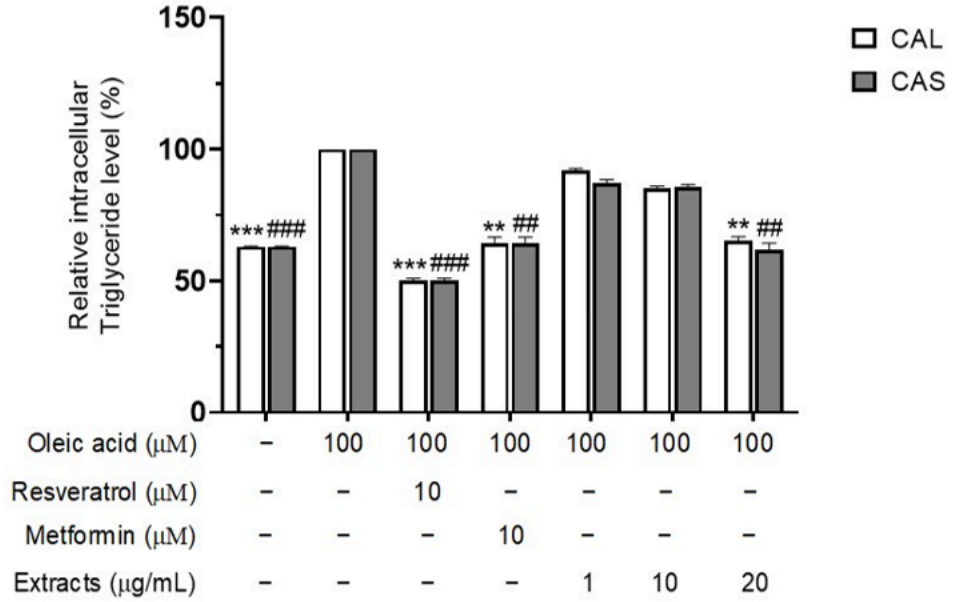

Figure 6

Effect of *C. afer* leaf (A) and stem (B) extracts on SW 872 and HepG2 cells ROS generation. Cells were incubated for 24 h with extracts. Data are expressed as % of control (H_2O_2) taken as 100; mean SD, N=3. *p < 0.05, **p < 0.01, and ***p < 0.001 vs. H_2O_2 control group in HepG2 cells. #p < 0.05, ##p < 0.01 and ###p < 0.001 vs. H_2O_2 control group in SW 872 cells; CAL: C. afer leaves, CAS: C. afer stems. "–": Absent.

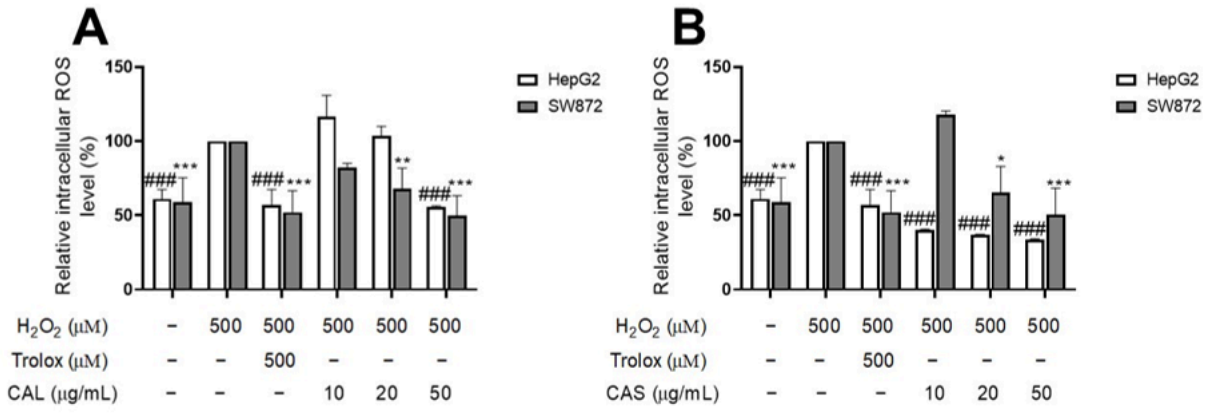

Figure 7

Effect of leaves (CAL) and stem (CAS) of *C. afer*extracts on triglyceride accumulation in differentiated SW 872 cells. Cells were incubated for 24 h with extracts. Data are expressed as % of control (differentiated SW 872 cells) taken as 100; mean SD, N=3. *p < 0.05, **p < 0.01, and ***p < 0.001 vs. CAL differentiated control group. ##p < 0.01 and ###p < 0.001 vs. CAS differentiated control group control group; CAS: *C. afer* stems; CAL: *C. afer* leaves. "–":

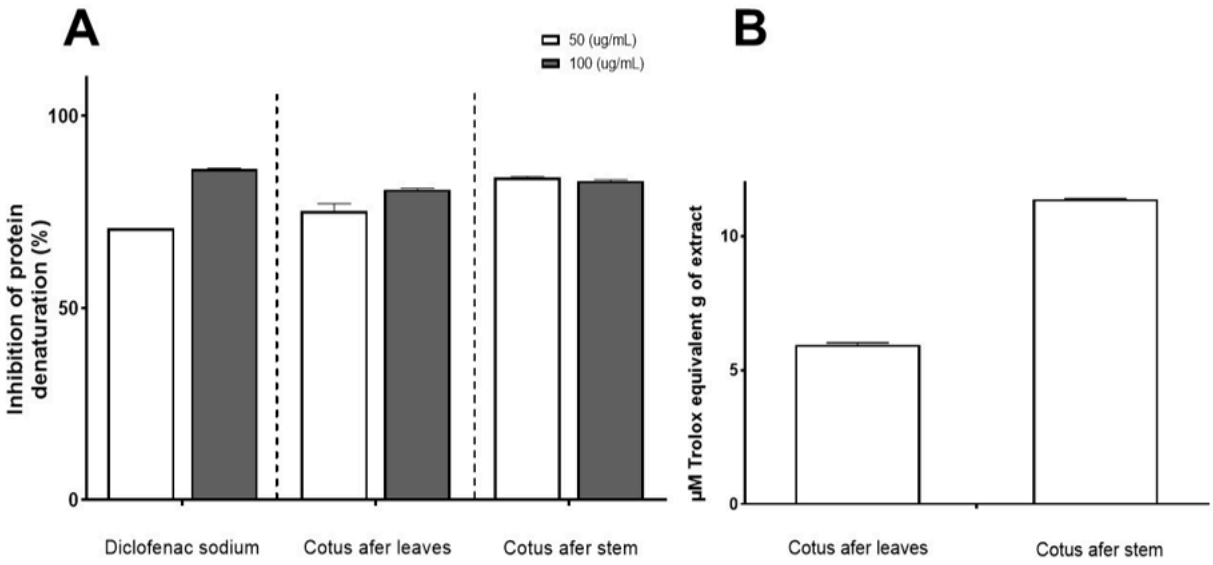

Figure 8

Inhibitory protein denaturation (A) and antioxidant (B) effect of *C. afer* extracts. Antioxidant activity is expressed as µmol Trolox equivalent Data reported in panel A are expressed as percentage (%); mean SD, N=3.

Supplementary Files

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