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Research Article

PROTECTIVE EFFECT OF BIOACTIVE FRACTIONS OF C. DALZIELII AGAINST WEIGHT GAIN IN MICE FEED WITH HIGH FAT-DIET

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ABSTRACT

Background**:* Obesity is an abnormal weight gain, this pathology is most often associated with diseases such as hypertension, heart failure, type II diabetes, insulin resistance, dyslipidemia, some cancer. *Caralluma dalzielii* is a medicinal plant traditionally used in northern Burkina Faso for weight management. ***Aim: The purpose of the study was to determine the ability of fractions of *C. dalzielii* to reduce weight gain. ***Methods:*** Acute oral toxicity of the raw extract of C. dalzielii was determined at 2000 mg / kg body weight. The activity of the fractions obtained by liquid-liquid fractionation on the digestive enzymes, on the weight gain and food of the animals having a hyperlipidicdiet, on the biochemical parameters related to obesity was determinated. ***Results**:* *Caralluma dalzielii* showed no acute toxicity at 2000 mg / kg body weight. For inhibition of digestive enzymes, the butanolic fraction gave the best inhibition with 88.47 ± 1.40% at 100 µg / ml on pancreatic lipase and the hexane fraction presented the best inhibitory effect of trypsin with 60.64 ± 1.75%. The Acetate ethyl fraction gave the best activity on reduction of body weight with 18.11% reduction at 50 mg / kg against the control 2 (hyperlipidicdiet) which gave weight increases of 34.83%. The ethylacetate fraction of *C. dalzielii* gave the best reductions in triglyceride. ***Conclusion**:* In vivo and in vitro biological activities, bioactive fractions of *C. dalzielii* contribute to reduce weight gain. Supplementation to these fractions may reduce the risk of complications related to obesity.

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INTRODUCTION

Obesity is a chronic and progressive disease. It is defined as a chronic condition characterized by an excess of body fat which causes an increase in the body weight of the patients. Its prevalence is constantly increasing throughout the world. In 2016, more than 1.9 billion adults aged 18 and over were overweight. On this total, more than 650 million were obese. Also, 41 millions children under 5 year old were overweight or obese (Who, 2018). In fact, overweight and obesity contribute to the development of serious pathologies such as type 2 diabetes and cardiovascular diseases, cancers that represent a real public health problem in many countries. Overweight and obesity can be estimated through Body Mass Index (BMI) which is a simple measure of weight versus height. Overweight is when the BMI is 25 or higher and is called obesity when the BMI is 30 or higher (Wennert, 2012). There are several pharmacological substances available as anti-obesity drugs, but they have dangerous side effects and so natural products have been used to treat obesity in many Asian countries (Matson, *et al*., 2012). The potential of natural products for the treatment of obesity is still largely unexplored and can be an excellent alternative to the safe and effective development of anti-obesity drugs (Bhutani *et al*., 2007)**.** In Burkina Faso, many medicinal plants are used both in urban and rural areas by people for the treatment of obesity and other related metabolic diseases. *Caralluma dalzielii* is one of the many species used to treat obesity in Burkina Faso. The genus Caralluma is known for its richness in pregnane glycosides, flavonoid glycosides, pregnane steroids, and β-sitosterol (Dutt *et al*., 2012). Pregnant glycosides are molecules that have recently shown anti-cancer, anti-obesity and appetite suppressant activities (Halaweish *et al*., 2004).

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The purpose of this study is to determine the protective effect of bioactive fractions of *C. dalzielii* against weight gain.

METHODS

Plant Material and extraction

The whole plant of *Caralluma dalzielii* was harvested in Gorom-gorom (Burkina Faso) (12°29'42.7 N, 1°24'1.2 W) in March 2018. The specie was authenticated and a herbarium was deposited in the UFR / SVT under identification codes ID-17052. Samples were dried under laboratory conditions away from the sun and then pulverised and stored in freezer bags for extractions. The powder (50 g) of plant material *Caralluma dalzielii* was placed in bottles containing 500 ml of absolute ethanol. The bottles were subjected to mechanical stirring for 24 heure at room temperature. The macerated were filtered and then concentrated in an evaporator equipped with a vacum pump and then evaporated to dryness. These extracts preserved and used for different tests

Fractionation of extract (liquid-liquid)

The ethanolic extract is fractionated using a series of solvents with increasing polarity hexane, dichloromethane, ethyl acetate and butanol, using a separating funnel. The crude extract is initially dissolved in water and then mixed with hexane (V / V), after decantation the upper organic phase is recovered. This step is redone three times. The hexane is subsequently evaporated under reduced pressure to dryness at 40 ° C by a rotary evaporator, and the resulting extract is considered to be the hexane fraction (FH). The lower aqueous phase is subjected to further fractionation by the dichloromethane, ethyl acetate and butanol to give respectively the dichloromethane (FDCM), ethyl acetate and butanolic fractions following the same steps as the first fractionation with hexane. The resulting raffinate represents the residual aqueous fraction (FAQ). The solvents were removed under reduced pressure and the concentrates were dried.

The acute toxicity of plant extracts

The toxicity was determined according to the method described by OCDE, 2001**.** The animal (mice) were randomised into batches of 6 mice (females). The animals were pre-fasted for 12 hours, then the weight of each mouse was taken, and they received a batch dose of extract. The route of administration of the extracts was oral, Signs of toxicity (writhing, panic, moribund state, death) were noted by batch after 2 h, 24 h, 48 h, 72 h and the animals were kept under observation for two weeks.

Phytochemistry of extracts

Total phenolics dosage

Total polyphenols were determined using Folin Ciocalteu reagent (Singleton *et al*., 1999). A volume of 125 µL of ethanol extracts (100µg/mL) was mixed with 625 µL of Folin-Ciocalteu reagent (0.2 N). After 5 min, 500 µL of aqueous sodium carbonate (Na_2CO_3, 75 g/l) were added. After 2 h of incubation in the dark at room temperature, the absorbances were measured at 760 nm against a blank by using a spectrophotometer. The experiments were carried out in triplicate. A standard calibration curve was plotted using gallic acid (0-50 µg/mL). The results are expressed in milligrams of gallic acid equivalent per 1 g of dry extract (mg EAG / 1 g).

Total flavonoids dosage

The total flavonoid contents of the extract were determined by the colourimetric method Arvouet-Grand *et al*., 1994). A volume of 75 µl of 2% AlCl3 in pure methanol was mixed with an equal volume of 1 mg/ml extract in methanol. The optical densities were read after 10 min at 415 nm using the spectrophotometer. Quercetin (0-100 mg / L) was used as a standard for the development of the standard curve. A mixture of 75 µl of extract and 75 µl of methanol without $AlCl_3$ served as a blank. In total, three (3) analyses were performed for extract and the result given was an average of the three readings. The results are expressed in milligrams equivalent quercetin for 1 g of dry extract (mg EQ / 1 g)

Determination of triterpenic compounds

The total triterpene content was evaluated according to the method described by Chang *et al*. 2012. A volume of 300 µl of vanillin-glacial acetic acid (5%) is mixed with 200 µl of the extracts dissolved in 100% methanol (final concentation 1 mg / ml); 1 ml of perchloric acid is added and all incubated at 60 degrees for 45 minutes. 4.5 ml of glacial acetic acid are then added and the reading is made at 548 nm with the spectrophotometer. In total, three (3) analyzes are performed for each extract and the result given is an average of the three readings. The results are expressed in equivalent milligram of ursolic acid per milligram of dry extract (mg E.A.U./mg)

HPLC-UV analysis of plant extracts

The analysis of the extracts was done on an Agilent 1100 HPLC equipped with a degasser, a pump and a diode array detector. A reversed phase (Spherisorb-Amino, 5µm) column was used for the separation of the compounds. The eluent consists of a mixture of acetonitrile and ultrapure water (80/20 v / v). The detection of these compounds was made at 254 nm over a 10-minute program with an injection volume of 5 µl. Seven phenolic compounds (caffeic acid, rutin, ellagic acid, quercetin, kaempferol and apigenin, catechin) were used as reference compounds for carrying out the analysis.

Digestive enzyme inhibition activities

Pancreatic lipase inhibition

The activity of pancreatic lipase performed according to the method defined by Changhyun *et al*., 2012. The activity is measured using p-nitrophenyl butyrate (p-NPB) as the substrate. Swine pancreatic lipase solutions (1 mg / ml) are prepared in 0.1 mM potassium phosphate buffer (pH 6.0) and the solutions are stored at -20 ° C. To determine the inhibition activity of lipases, extracts (final concentration of 100 µg / ml) or Orlistat as a positive control were pre-incubated with porcine pancreatic lipase for 1 h in potassium phosphate buffer (0.1 mM, pH 7.2) at 30 ° C before assaying for porcine pancreatic lipase activity. The reaction then began following the addition of 0.1 µl pNPB as substrate, all in a final volume of 100 µl. After incubation at 30 ° C for 5 min, the amount of p-nitrophenol released in the reaction was measured at 405 nm using a UV-Visible spectrophotometer. Negative control activity was also examined with and without inhibitor. Inhibitory activity (I) was calculated according to the following

formula: Percent inhibition (I%) = 100 - ((B - b) / (A - a) × 100)

Where: A represents the activity without inhibitor, a is the negative control without inhibitor, B is the activity of the inhibitor, and b is the negative control with an inhibitor.

Trypsin inhibition

The activity was carried out according to the method described by Arefrad *et al*. in 2013. To measure the inhibitory activity of extracts on trypsin, 5 µg of trypsin (from bovine pancreas, Sigma) and 5 µg of total protein extract were combined in a suitable volume of 0.2 M Tris-HCl pH 7, 8 to obtain a total volume of 800 µl of enzyme solution. The trypsin and the extract were incubated for 5 min before the addition of 160 µg of p-nitroanilide N-α-benzoyl-DL-arginine in 200 µl of substrate buffer (0.05 M Tris-HCl pH 8.2, 0.05 M CaCl2). The released p-nitroanilide was monitored for 25 min at 410 nm using a spectrometer. The percentages of inhibition were calculated by the following equations:

Percent inhibition (%) = 1 - (OD Extract / DO Enzyme) $x100$
OD Extract = Optical density of the extract
DO Enzyme = Optical density of the enzyme

Evaluation of the anti-obesity potential on animals with hypercaloric feeding

Animal treatment

The animals were divided into five (5) groups of six (6) mice each. A series of three doses (25, 50, 75 mg/kg body weight) of extract fraction preparation was administered to the different groups of animals, with a negative control group that received only the vehicle (water) plus the standard food and a positive control that received the vehicle and hyperlipidic food. The extracts were administered for a volume not exceeding 200 µl. The animals were placed 12 hours in the light; 12 hours in the dark and they had free access to food and water. They were treated for 30 days (Mei-Yin *et al*., 2016).

Group 1: Standard Food
Group 2: Hyperlipidic Food
Group 3: Hyperlipidic Food + 25 mg / kg body weight of the extract
Group 4: Hyperlipidic Food + 50 mg / kg body weight of the extract
Group 5: Hyperlipidic Food + 75 mg / kg body weight of the extract

Hyperlipidic diet Composition: protein (26%), corn starch (15%), sucrose (9%), lard (40%), cellulose (5%), mineral mixture and vitamin (5%).

Anorectic activity of the extract (food intake)

It consisted to determined the effect of extract on the amount of food taken from the animals. The objectif was to measure the total amount of food remaining per weigh each day for each batch. Food consumption = total amount of food given to the animal - amount of food remaining

Slimming potential of the extract (weight gain)

Determination of body weight: During the fourth weeks of the study period, the body weight of each animal was measured every three days using a standard weighing device. The net weight gain was calculated as follows: Net weight gain = final weight - initial weight

Serum biochemical parameters analyzes (AST (ALT, cholesterol, total triglycerides, LDLc)

At the end of the fourth week of treatment the animals are sacrificed after being anesthetized with ketamine (150 mg / kg of weight). The blood of the animals was collected by cardiac puncture in dry tubes, centrifuged at 3000 rpm for 5 minutes and the serum were taken in order to evaluate the enzymatic parameters and the rate of lipid indices such as Aspartate Amino Transferase (AST), Alanine Amino Transferase (ALT), cholesterol, total triglyceride, LDLc by colorimetric using commercial Kits.

Antioxidant activity of the liver (cat, sod, mda)

Lipid peroxidation (Dosage of MDA)

Inhibitory activity of lipid peroxidation (LPO) extract or fractions were determined by the method of 2-thiobarbituric acid (Ohkawa, 1979). Iron dichloride ($FeCl_2$) with hydrogen peroxide H_2O_2 was used to induce peroxidation of rat liver homogenate. In this method 0.2 mL of the extracts (1.5 mg / mL) was mixed with 1.0 mL liver homogenate in 1% Tris-HCl buffer (50Mm, pH 7.40), 50 µL $FeCl_2$ (0.5 mM) and 50 µL of H_2O_2 (0.5 mM) were added. The mixture was incubated at 37° C for 60 minutes, then 1 mL of trichloroacetic acid (15%) and 1 mL of 2-thiobarbituric acid (0.67%) were added and the mixture is heated in boiling water for 15 minutes. Absorbances were read at 532 nm using the spectrophotometer. The percentage inhibition of lipid peroxidation is calculated according to the formula: % Inhibition = $((\boldsymbol{A}0-\boldsymbol{A}1)/\ \boldsymbol{A}0) \times 100$
With A0 the percentage of inhibition of the negative control, A1 the absorbance of the sample. Quercetin is used as a positive control.

Catalase measurement

Activity of catalase is tested using the standard method given by Beers and Siezer 1952; The degradation of H_2O_2 by addition of the enzyme is followed by absorption of light. The absorption of the peroxide solution in the UV region is determined. A reaction mixture of 3 ml containing 1.9 ml of phosphate buffer (0.05 M, pH 7) .1.0 ml of H_2O_2 substrate (30 mM) and 0.1 ml of diluted enzyme was used in these assays. Activity was measured as a change in optical activity at a density of 240 nm at one minute intervals for about 3 minutes. The activity of catalase was expressed in terms of µmol of consumed H_2O_2 / min / mg of protein.

Measurement of superoxide dismutase (SOD)

Activity of SOD was tested by the standard methods revealed by Misra *et al*., 1972. It is based on the inhibition of the transition of epinephrine adrenochrone by the enzyme .0.5 homogeneous plasma was collected in a container of reaction then 0.5 ml of distilled water to dilute the sample. 0.25 ml ice-cold ethanol and 0.15 chloroform was added to precipitate the reaction mixture. The reaction mixture is stirred well for about 5 minutes at 4 degrees C. Then centrifuged. The adrenochron produced in the reaction mixture contains 0.2 ml of EDTA (0.6 mM), 0.4 ml of Na_2CO_3 (0.25 M) and 0.2 ml of epinephrine (3 mM), the volume Final was adjusted to 2ml (0.3ml distilled water) and absorbance were measured at 420nm in a UV-Visible spectrometer. The transition from epinephrine to

adrenochrome was determined by the addition of the required amount of enzyme to evaluate the enzymatic activity expressed in units / minute / mg protein.

Inflammatory enzyme inhibition activity tests

Inhibition test of xanthine oxidase (XO)

The inhibitory activity of extracts on XO (EC.1.1.3.22) was evaluated according to the method described by Filha *et al*. (2006). The reaction mixture consists of 50 µL of extract or fraction at the final concentration of 100 µg / mL, 150 µL of phosphate buffer (pH 7.5, 1/15 M) and 50 µL of enzyme solution (0 28 U / mL prepared in buffer). After preincubation of the mixture at 25 ° C for 1 min, the reaction is initiated by adding 250 µl of a substrate solution (0.6 mM) and the absorbance is measured for three minutes. A blank is prepared without extract. Quercetin and gallic acid are used as positive controls. The inhibitory activity of xanthine oxidase, expressed as percent inhibition, is calculated using the formula below:

Percentage of inhibition (%) = $(1 - V/V0)\ x100$

V0: variation of the absorbance per minute of the test without the extract; V: variation of the absorbance per minute of the test with the extract.

Inhibition test of lipoxygenase

The inhibitory activity of extracts and fractions on lipoxygenase was determined by the spectrophotometric method developed by Malterud *et al*., (2000). Briefly, the reaction medium consisted of a mixture of 100 µL of extract or fraction prepared in borate-methanol buffer (1%) and 400 µL of LOX (167 U mL-1). The mixture was incubated at room temperature for 2 minutes and the reaction is initiated by adding 500 µl of the substrate solution (linoleic acid, 250 µM in borate buffer). The kinetics of the reaction are monitored at 234 nm for 2 min. The inhibitory activity, expressed as percentage inhibition of lipoxygenase is calculated as follows:
Percentage of inhibition (%) = $(1 - B/A)\ x100$

A = activity of the enzyme without inhibitor (Δabs with enzyme-Δabs without enzyme) B = activity of the enzyme with inhibitor (Δabs with enzyme-Δabs without enzyme)

Tests d'activités antioxydants

Inhibition of the radical DPPH. (2,2diphenyl-1-picrylhydrazyl)

The anti-radical activity of the ethanolic extract (1 mg/ml) was evaluated by the DPPH (2,2diphenyl-1-picrylhydrazyl) method **(**Velazquez, 2003)**.** This method is based on the reduction in absorbance at 517 nm of the stable free radical DPPH, in the presence of a hydrogen radical donor, three (03) tests were carried out by mixing 100 µl of the sample and 200 µl of DPPH (20 mg / l in methanol). After 15 minutes of incubation, the absorbance is read at 517 nm against a blank using a spectrophotometer. Quercetin and gallic acid were used as reference substances. The antiradical activity was expressed in percent inhibition

Reducing power FRAP (Ferric reducing antioxidant power)

The FRAP method is based on the reduction of ferric ion (Fe^{3+}) to ferrous ion (Fe^{2+}) by the reducing compounds following an electron mono electron transfer (Hinneburg, 2006)**.** In test tube containing 0.5 ml of extract (1 mg / ml), 1.25 ml of phosphate buffer (0.2 M, pH 6.6) and 1.25 ml of potassium hexacyanoferrate (1% aqueous) were added. The mixture was heated at 50°C in a bain-marie for 30 minutes. After cooling, trichloroacetic acid (1.25 mL, 10%) was added, and the mixture was then centrifuged (2000 rpm for 10 minutes). Three aliquots (125 µl) of the supernatant were transferred into a 96-well microplate to which 125 µl of distilled water and then 25 µl of $FeCl_3$ (0.1% aqueous) were added. The reductive power was evaluated at 700 nm using a spectrophotometer. The experiment was carried out in triplicate (independent tests), and the reducing activity of the extract was expressed in mmol Equivalent Ascorbic acid per gram of extract (mmol EAA / g extract). Quercetin and gallic acid were used as reference substances.

Inhibition of deoxyribose degradation

The ability of the plant extract to trap the hydroxyl radical was evaluated using the deoxyribose degradation scavenging assay as described by Perjési *et al*., (2011)**.** The reaction mixture was constituted by 100 µl of extract (1 mg / ml in 50 mM phosphate buffer, pH 7.4), 100 µl of EDTA (1.04 mM aqueous), 100 µl of iron sulfate (100 µl aqueous mM), 100 µL of deoxyribose (60 mM aqueous) and 100 µL of hydrogen peroxide (10 mM). The volume was made up to 1 mL with phosphate buffer, and the mixture was incubated (37 ° C for 1 hour). Trichloroacetic acid (1 mL, 15% aqueous) and thiobarbituric acid (1 mL, 0.675% in 25 mM aqueous NaOH) were added, and the whole was then incubated (100 ° C. for 15 minutes). After cooling in an ice bath (5 minutes), the tubes were centrifuged (3000 rpm for 10 minutes) and then 200 µL of the supernatant were transferred to 96-well microplates. Trapping of deoxyribose degradation was measured spectrophotometrically at 532 nm against a blank. Quercetin has been used as a reference substance. The activity of the extract to trap deoxyribose degradation was expressed as a percentage of deoxyribose degradation trapping compared to control without extract.

Inhibition of hydrogen peroxide

Hydrogen peroxide has a relatively long life and can cause damage far from its place of production. It diffuses easily through the cell membrane. The ability of the plant extract to trap hydrogen peroxide was evaluated according to the method described by Mohan *et al*., (2012). The reaction mixture consisting of 100 µl of extract (200 µg / ml in 10 mM phosphate buffer, pH 7.4) and 100 µl of hydrogen peroxide (100 mM) was incubated for 10 minutes at room temperature. Residual hydrogen peroxide was measured at 230 nm against a blank containing only the phosphate buffer. The activity of the hydrogen peroxide trapping extract was expressed as a percentage of trapping hydrogen peroxide relative to the control without extract. Gallic acid was used as a reference substance.

Statistical Analysis

All results were expressed as the mean value of several independent experiments (n = 3) ± standard deviation. For statistical analysis, Graph Pad Prism software (version 5.0) and MS Excel software were used to obtain standard curves and graphs, percentages of inhibition, averages and standard deviations. Anova one way followed by the Tukey test was

used to measure the degree of statistical significance of the results. A significant difference was considered for P<0.05.

RESULTS

Toxicity

On toxicity we did not observe mortality or signs of intoxication after seventy-two hours (72 H) of observation following the administration of *C. dalzielii* extracts on batches of six (06) mice (table1).

Table 1 Toxicity of extracts

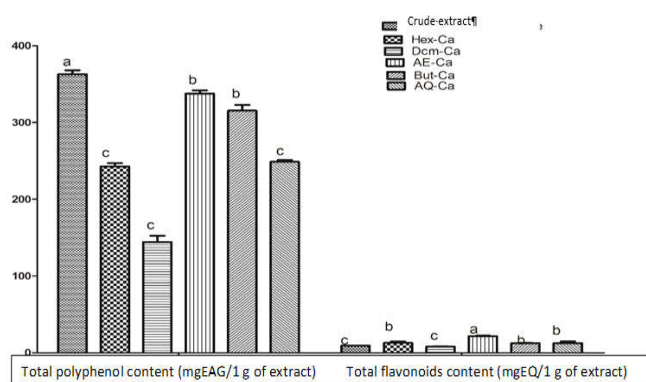
Plante	Dose	Number of mice used	Number of deaths after				% of death after 72h	Signs of toxicity
			2h	24h	48h	72h		
C. dalzielii	2000 mg/kg.pc	06	00	00	00	00	00	No sign of toxicity

Phytochemistry of extracts

Phenolic compounds in extracts

Total flavonoids and total polyphenols were quantified against quercetin and gallic acid curves. The results are shown in the graph.1.

The crude extracts of *C. dalzielii* showed the highest polyphenol content with 368.875 ± 16.2 mg EAG / g compared to the fractions of the extract. The ethyl acetate fraction gave the best content of total flavonoids : 21.57 ± 0.74 mgEg / g of extract.

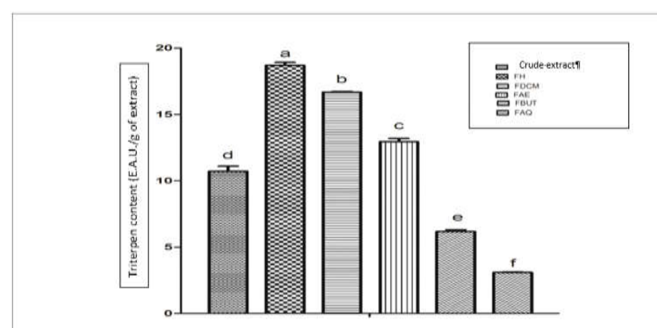


Graph 1 Content of phenolic compounds

HEX-= hexanic fraction of *Caralluma dalzielii*; DCM= dichloromethanic fraction of *Caralluma dalzielii,* AE= ethyl acetate fraction of *Caralluma dalzielii*, BUT = butanolic fraction of *Caralluma dalzielii*, AQ = aqueous fraction *Caralluma dalzielii*, CD= *Caralluma dalzielii* Mean ± S.E.M = Mean values ± Standard error of means of three experiments. Values with different superscript letters (a, b, c, d, e, f) differ significantly (P < 0.05) for each measured parameter

Teneurs en composés triterpéniques

Total triterpenoids were quantified using a standard urosolic acid curve. The results are shown in the graph 2. The hexanic fraction gave the best triterpenoid content with 18.70 ± 0.37 mg E.A.U / mg extract compared to the crude extract and other fractions.



Graph 2 Content of triterpenic compounds

HEX-= hexanic fraction of *Caralluma dalzielii*; DCM= dichlorométhane fraction of *Caralluma dalzielii*, AE= ethyl acetate fraction of *Caralluma dalzielii*, BUT = butanolic fraction of *Caralluma dalzielii,* AQ = aqueous fraction of *Caralluma dalzielii, CD= Caralluma dalzielii.* Mean ± S.E.M = Mean values ± Standard error of means of three experiments. Values with different superscript letters (a, b, c, d, e, f) differ significantly (P < 0.05) for each measured parameter

Identification of phenolic acids and flavonoids by HPLC-UV of plant extracts

In this study seven reference phenolics were searched in ethanolic extracts of *C. dalzielii* through HPLC-UV. The chromatograms of the compounds showed different retention times: quercetin at 5.89 min, kaempferol at 2.34 min, caffeine at 3.55 min, rutin at 5.22 min, ellagic acid at 6.69 min, catechin at 2.62 min and apigenin at 2.30 min

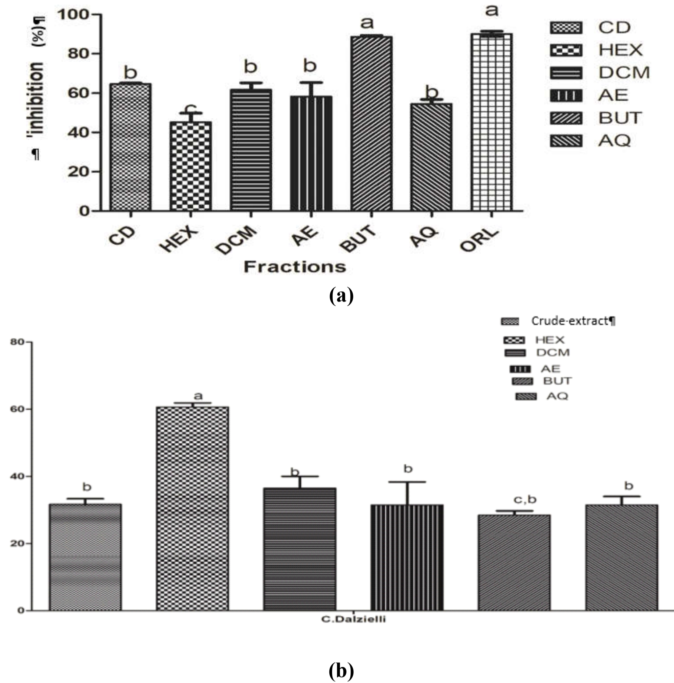
Table 2 Results of HPLC analyse

Molecule	Quercetin	Kaempferol	Cafein	Rutin	Ellagique acid	Catechin	Apigenin
Retention time (RT)	5,89 Min	2,34 Min	3,55 Min	5,22 Min	6,69 Min	2,62 Min	2,30 Min

Analysis of the chromatographic spectra of the ethanolic extract of *C. dalzielii* showed the presence of kaempferol (RT = 2.25 min), quercetin (RT = 5.90 min) in *C. Dalzielii*

In vitro anti-obesity activities of fractions

The inhibitory activities of the ethanolic extract of *C. dalzielii* against porcine pancreatic lipase are shown in Graph 3 (a). The extract of *C. dalzielii* and these fractions Hex, AE, DCM, BUT, AQ respectively inhibits the pancreatic lipase activity of 45.20 ± 7.9; 42.78 ± 1.07; 61.68 ± 6.17; 88.47 ± 1.40; 54.58 ± 3.85% at 100µg / ml. Orlistat, a standard inhibitor of pancreatic lipase used as an anti-obesity agent, inhibits the enzymatic activity of 90,15 ± 2,2 at a concentration of 10 µg / ml. The butanolic fraction of *C. dalzielii* exhibited the best inhibitory activity of the extracts with a non-statistically different value from that of the Orlistat reference product (P <0.05). Graph 3 (b) shows the inhibitory potency of plant extracts and their fractions. For the *Caralluma dalzielii* extract the hexanic fraction showed the best trypsin inhibitory potency with 60.64 ± 1.75%, the butanolic and acetate d'ethyl fractions have been used for the verification of their anti-obesity potential in vivo.



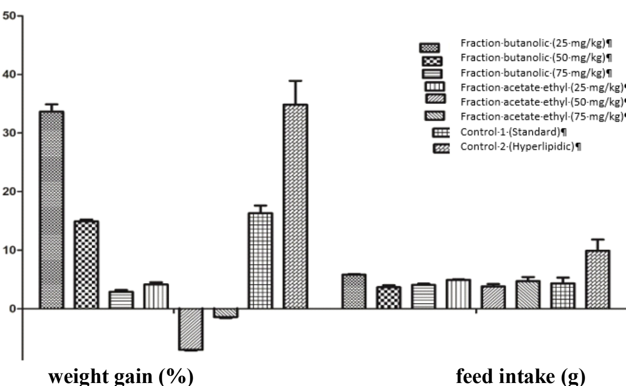
Graph 3 Effect of extract on pancreatic lipase and trypsin activities

HEX-= hexanic fraction of *Caralluma dalzielii*; DCM= dichlorométhane fraction of *Caralluma dalzielii*, AE= acetate ethyl fraction of *Caralluma dalzielii*, BUT = butanolic fraction of *Caralluma dalzielii,* AQ = aqueous fraction of *Caralluma dalzielii, CD= Caralluma dalzielii*. Mean ± S.E.M = Mean values ± Standard error of means of three experiments. Values with different superscript letters (a, b, c, d, e, f) differ significantly ($P < 0.05$) for each measured parameter

Anorexigenic activity and slimming potential in vivo of bio-actives fractions

The anti obesity potential in vivo of the fractions having the best polyphenol and flavonoid contents as well as the best anti-obesity activity in vitro.

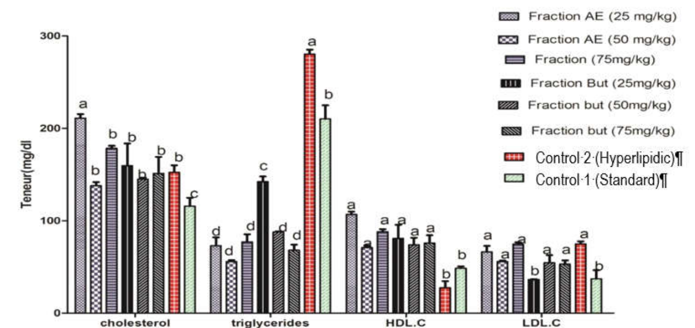
Graph 4 shows the effect of butanolic (But) and ethyl acetate (EA) fractions on weight and feed intake of treated animals. The AE fraction of *C dalzielii* gave the best activity at a rate of 50 mg / kg compared to control 1 (standard diet) and control 2 (hyperlipidic diet), which gave weight increases respectively 16.61% and 34.83%. The butanolic fraction showed the lowest consumption at 50 and 75 mg / kg. The hyperlipidic diet is applied to animals induced weight gain.



Graph 4 Effect of fractions on weight and food intake

Analyzes of serum biochemical parameters

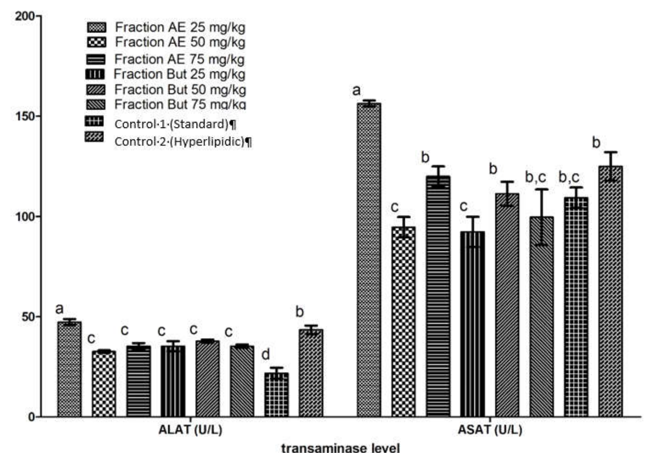
Graph 5 shows the effect of fractions on the serum cholesterol, triglyceride, HDLc, and LDLc levels of the treated animals. Fractions had no effect on cholesterol levels. For the triglyceride level the fractions have considerably reduced this rate in the treated animals; the ethyl acetate fractions of *C. dalzielii* gave the best reductions in triglyceride level with 73.07 ± 5.4; 55.89 ± 2.40; 77.15 ± 4.3 mg / dl for the doses of 25, 50, 75 mg / kg respectively against the control (only the hyperlipidic diet) which gave a rate of 280 ± 8.43 mg / dl. The high lipid diet reduced the level of HDLc and the treatment of obese animals by the fractions increased this level. The fractions showed a regulatory effect on the HDLc level. The hyperlipidic diet had no effect on the HDL level in the presence of the fractions. The butanolic fractions of *C. dalzielii* induced a reduction in LDLc or bad cholesterol levels compared with controls.



Graph 5 Effect of extract fractions on serum parameters

Mean ± S.E.M = Mean values ± Standard error of means of three experiments, Values with different superscript letters (a, b, c, d, e, f) differ significantly ($P < 0.05$) for each measured parameter.

Serum transaminase levels (ALT, AST) have always been considered as sensitive markers of liver damage. Butanolic (50 mg / kg) and ethyl acetate (25 mg / kg) fractions of *C. dalzielii* gave a significant (at $p < 0.05$) reduction in serum of ALT levels in treated animals compared to batch control having a hyperlipidic diet (Graph 6). Ethyl acetate fraction (25, 50 mg / kg) and butanolic (25 mg / kg) gave the best activities for the reduction of ASAT in treated animals compared to hyperlipid control.



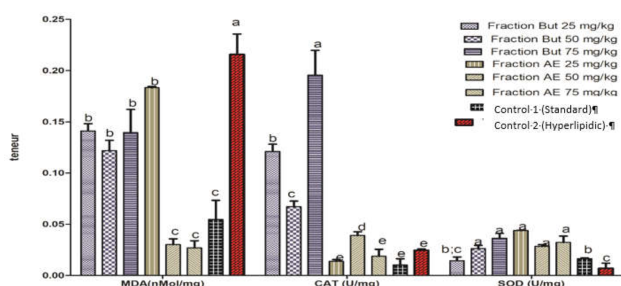
Graph 6 Effect of fractional extracts on transaminases

ALAT= Alanine aminotransferas, ASAT= aspartate aminotransferas

Mean ± S.E.M = Mean values ± Standard error of means of three experiments. Values with different superscript letters (a, b, c, d, e, f) differ significantly (P < 0.05) for each measured parameter

Antioxidant activity of the liver

The potential of plant extract fractions to reduce hepatic oxidative stress was evaluated by their action on superoxide dismutase, catalase, MDA. The hyperlipidic diet showed an increase in MDA levels, a reduction in the SOD and catalase content in the animals not treated with the extracts (Graph 7). The anti-oxidant effect of the fractions is characterized by a reduction of MDA level and an induction of SOD and catalase production. The AE fraction (50 and 75 mg / kg of weight) gave the best reduction in the MDA content, the butanolic fractions at 25, 50, 75 mg / kg gave the best enhancement of the catalase. For SOD test, AE and But fractions showed an induction of SOD production compared to the control.



Graph 7 Effect of extract fractions on liver anti-oxidant parameters

MDA= Malondialdehyde, Cat= Catalase; SOD= Superoxide dismutase, Fraction AE= acetate ethyl fraction, Fraction but= butanolic fraction**.** Mean ± S.E.M = Mean values ± Standard error of means of three experiments. Values with different superscript letters (a, b, c, d, e, f) differ significantly (P < 0.05) for each measured parameter

Inhibitory Activities of Inflammatory Enzymes and Antioxidant Activities

The fractions showed good anti-inflammatory activity, indeed the AE fraction of *C. dalzielii* showed an inhibition of lipoxygenase greater than that of gallic acid. Both fractions gave xanthine oxidase inhibition greater than 50% but less than that of gallic acid used as reference. Against the hydrogen peroxide both fractions had a higher inhibition than the gallic acid used as reference substance. For the inhibition of the DPPH radical, deoxyribose degradation, reduction of ferric ion, the extracts showed good antioxidant activity (Table 3)

greater than 2000 mg / kg body weight are very low in toxicity. So, extracts of *C. dalzielii* have very slight acute toxicity. Tanko *et al*., 2012 also found an LD_{50} above 2000 mg / kg body weight for *C. dalzielii* extract. Obesity is a chronic condition characterized by excess body fat that results in increased body weight (seidell *et al*., 1997). This metabolic disorder results from an imbalance between energy intake (food) and that spent on physical activity (Woods *et al*., 1998)**.**

Quantitative assays have shown that the total polyphenol, total triterpenes and total flavonoid contents of the fractions are interest. Some flavonoids are known to reduce obesity. Indeed, resveratrol (Stilbenoid) and genistein (Isoflavone) produce a reduction in weight by their inductive action of apoptosis of adipocytes (Srujana *et al*., 2008). Many terpene compounds are known for their anti-obesity activity. Celastrol, a pentacyclic triterpene extracted from the roots of *Tripterygium wilfordi* is a potent anti-obesity agent, indeed this molecule suppresses food intake, blocks the reduction of energy expenditure by increasing sensitivity to leptin (Liu *et al*., 2015). The different antiobesity molecules act on several levels of the body to lead to weight reduction.

Pancreatic lipase and trypsin are two enzymes involved in the digestion of lipids and proteins respectively (Veeramachaneni *et al*., 2015, McDougall *et al*., 2005). Their inhibition will reduce the absorption of nutrients in the body. The butanolic fraction of extracts of *C. dalzielii* having the best activity at 100 µg / ml, not statistically different from that of orlisat which is a reference molecule used in the composition of an anti-obesity drug; this fraction would be rich in substances that inhibit lipase. The results corroborate those of Young *et al*. in 2013 who also found a butanolic fraction of *Polygonum cuspidatum* more active on pancreatic lipase. De-Leoa *et al*. in 2005 have indeed isolated pregnans glycosylated in this plant and which could be responsible for the weight reduction observed in animals with hyperlipidic diet and treated with extracts; the acetate ethyl fraction could be rich in pregnane glycosides which would explain the best activity of the fraction. Trypsin inhibitors work by reducing food intake and weight gain by increasing the rate of neurohormone CCK. CCK is an anorectic hormone that sends satiety signals to the brain. Indeed, the activity of these medicinal species of plants is certainly linked to the presence of secondary metabolites such as polyphenols, glycosed pregnanes, mannans who can reduce rate of the triglycerides, glucose, cholesterol or inhibit enzymes involved in lipid metabolism such as pancreatic lipase, lipoprotein lipase and glycerophosphate dehydrogenase or to increase energy expenditure.

Table 3 Antioxidant and Anti-inflammatory Activity of Fractions

	Anti inflammatory activity		Antioxidant activity			
	Inhibition of lipoxygenase (%)	Inhibition of xanthine oxydase (%)	DPPH (%)	Inhibition of deoxyribose (%)	Inhibition of hydrogen peroxide (%)	FRAP
AE Fraction	69,47 ± 8,02a	64,58 ± 2,94c	46,843 ±5,5c	70,5 ± 0,15c	25,39 ± 1,6b	0,88 ± 00,2d
But Fraction	51,72 ± 0,28c	64,58 ± 2,94c	59,181 ± 2,3	69, 44 ± 1,17c	20,6 ± 2,42	1,297± 0,05c
Gallic acid	54,87 ± 0,043c	90,90 ± 00 a	86,63±0,08	88,00 ± 0,07b	14,19 ± 0,70c	8,84± 0,45a
Quercetin	75,09 ± 8,21a			95,78 ± 0,03a		4,69± 0,05b

Fraction AE= acetate ethyl fraction, Fraction but= butanolic fraction, Mean ± S.E.M = Mean values ± Standard error of means of three experiments. Values with different superscript letters (a, b, c, d, e, f) differ significantly (P < 0.05) for each measured parameter.

DISCUSSION

Caralluma dalzielii extract showed no mortality at 2000 mg / kg body weight. According to WHO extracts with an LD_{50}

All these mechanisms result in a reduction of body fat and thus body weight. Acetate d'ethyl and Butanol fractions showed good weight reduction activity of the treated animals compared

to the animals feed with normal and and hyperlipidic food. These fractions could contain molecules that act on the mechanism of weight gain. The activity of the fractions on trypsin could explain the anorectic activity observed on the animals.

Obesity is accompanied by an increase in triglyceride, LDLc and a reduction in HDLc. The acetate ethyl fraction gave the greatest reduction of triglyceride in serum as compared to the butanol fraction and the control fed with a hyperlipidic diet without treatment with the extract that caused the greatest accumulation of triglycerides. The fractions generally showed an induction of HDLc production and inhibition of HDLc production. The activity of fractions on biological parameters related to obesity could explain the reduction of the weight of animals treated with these extracts.

Obesity is most often accompanied by high levels of triglyceride, MDA and LDLc followed by a reduction in HDLc as well as a reduction in the activity of antioxidant enzymes such as SOD, catalase (Alba *et al*., 2011). The acetate ethyl fraction of the plant at 50 and 75 mg / kg showed the best MDA reductions, indeed MDA is a product of lipid peroxidation which is found to be high during obesity (Misra, *et al*., 2009); the reduction of this rate observed with this fraction could explain a certain activity of this fraction.

The high calorie diet caused an increase in ASAT in untreated animals, which is corrected by the fractions. Obesity is most often associated with increased transaminases (Clark, *et al*., 2003). SOD and catalase are two antioxidant enzymes involved in the inhibition of reactive oxygen species (Favier, 2003). In this study the fractions showed an induction of synthesis of SOD and catalase at the hepatic level and that in the presence of a hyperlipidic diet, but these decreased in obese animals. This result probably explains the hepatic protection of animals treated with the extract.

It is recognized that obesity is associated with a chronic inflammatory state, characterized by abnormal production of pro-inflammatory cytokines (TNF-α and IL-6) and an increase in circulating levels of markers of systemic inflammation such as CRP. Cytokines produced during obesity can irreversibly convert xanthine dehydrogenase to the active form xanthine oxidase (Vorbach *et al*., 2003). Fat-rich diets leading to obesity activate 12-lipoxygenase (12-LOX) which catalyzes the oxygenation of cellular arachidonic acid to form pro-inflammatory intermediates (leukotrienes and lipoxins) (Tersey *et al*., 2014). Fractions of *Caralluma dalzielii* showed significant inhibitions of XO and LOX. These fractions could contain anti-inflammatory agents. Kong *et al*.,2001 have indeed shown the inhibitory activity of glycosides isolated from the extract of *Conyza bonariensis* on xanthine oxidase; also Bezakova *et al*., 1996 isolated LOX inhibitory glycosides in *Calendula officinalis* L. The inhibitory potentials observed in the extracts may be due to the glycosides contained in these species (Mohammed *et al*., 2000)**.**

Oxidative stress and inflammation are key factors in the pathogenicity of obesity-related diseases, such as type 2 diabetes, cardiovascular disease and cancer (Dandona *et al*., 2005).

The set of antioxidant methods (DPPH, inhibition of deoxyribose, hydrogen peroxide, reduction of ferric ion) indicated that the fractions have an interesting antioxidant power. Thus these extracts fractions contain substances that protect biological molecules including DNA, RNA, proteins and lipids. Some substances such as polyphenols are endowed with antioxidant properties : they are capable of trapping free radicals, inhibiting lipid peroxidation by reducing hydroxyl radicals, superoxides and peroxides. They are also able to trap metal ions because they have chelating properties (Delattre *et al*., 2005).

HPLC-UV showed kaempferol in both extracts; this molecule has anti-obesity properties by acting on adipocyte transcription factors (Zang *et al*., 2015). Quercetin in *C. dalzielii* exhibits anti-obesity properties by inhibiting lipid accretion in adipocytes (Edward *et al*. 2007) or by inducing apoptosis (Yamamoton *et al*. 2006). The presence of these molecules would justify the traditional use of this plant against obesity.

CONCLUSION

It was found that the crude ethanolic extract of *C. dalzielii* showed the best total phenol content and the ethyl acetate fraction showed the highest total flavonoid content. Butanol, hexane, and ethyl acetate fractions contain the terpenoic compounds. By in vivo biological activities, bioactive fractions of *C. dalzielii* would help to reduce weight gain induced by a high calorie diet, inhibit accumulation of serum lipids and preserve the integrity of the liver by stimulating its antioxidant system. Supplementation to these fractions may reduce the risk of complications related to obesity.

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