**Nutritional and bioactive potentials of powdered leaves of *Adansonia digitata* L., *Corchorus olitorius* L., and bark of *Triumfetta cordifolia* A. rich.: Effect on GLUT4 translocation activity**

**ABSTRACT**

The consumption of medicinal plants in the management of type 2 diabetes (T2D) is increasingly recommended by therapists. The leaves of *Adansonia digitata (LAD)*, *Corchorus olitorius (LCO)*, and the bark of *Triumfetta cordifolia* *(BTC)* are parts of the plants commonly consumed as traditional dishes for their nutritional and bioactive potential. However, very few studies have evaluated the effects of the constituents of these plants on GLUT4 translocation. The objective of this study was to determine the nutritional and bioactive composition of the powders from the *LAD, LCO*, and the *BTC,* as well as their effects on GLUT4 translocation activity. The *LAD, LCO*, and the *BTC* were processed into powders after harvest. Proximate composition, energy value, and mineral content were determined from the obtained powders. Bioactive compounds and their profiles, along with GLUT4 translocation activities, were evaluated from the extracts of the powders. It was found that the powders of *A. digitata* and *C. olitorius* had the lowest energy values of 257.75 ± 3.98 and 270.68 ± 1.65 Kcal/100g, respectively, and available sugar contents of 37.72 ± 0.45 and 38.24 ± 0.20 g/100g DM. However, only *C. olitorius* exhibited the highest total protein content of 21.60 ± 0.10 g/100g DM, with macroelement contents being the highest at 2200.00 ± 4.00; 1957.41 ± 3.89; and 580.00 ± 1.45 mg/100g DM for calcium, potassium, and magnesium, respectively. The powder of *T. cordifolia* had the highest total flavonoids of 4.90 ± 0.05 µg QE/g DM. Flavonoids such as isoquercetin and quercetin have effects on GLUT4 translocation from the extracts of the powders of *A. digitata* and *C. olitorius* of 90.61 and 92.52%Insulin, respectively. These activities are closely dependent on calcium content. The powder of *C. olitorius* demonstrates the best nutritional and bioactive potential and may be recommended for the management of T2D patients.

***Keywords:*** *Type 2 diabetes; Plant powders; Nutritional composition; Bioactive compounds; GLUT4 translocation*

1. **INTRODUCTION**

The high consumption of medicinal plants by populations is increasingly observed and encouraged by many therapists in Africa in general and in Cameroon in particular. This situation has worsened with the various health crises that have affected Africa, notably COVID-19, the measles epidemic, monkeypox (mpox), malnutrition, food insecurity, and the rising prevalence of chronic diseases (Abdel-Razek et al., 2022; Saddari et al., 2022). Many plant matrices commonly consumed as traditional dishes and used in various therapies have seen their prices double or even triple in markets. This situation has raised numerous concerns among researchers and health agents regarding the necessity to master the multiple potentials, whether nutritional, medicinal, or even therapeutic, of our plants (Assiéné, Djeukeu, Assiéné, Tize, et al., 2024; Assiéné, Djeukeu, Assiéné, Mbida-Mbida, et al., 2024). Among these are the leaves of *Adansonia digitata, Corchorus olitorius,* and the bark of *Triumfetta cordifolia*.

*A. digitata* L. (Bombacaceae), commonly known as baobab, is a tree found in the dry and hot regions of tropical Africa (Cameroon, Nigeria, Ghana, Ivory Coast, etc.). It is a massive and majestic tree that can reach over 25 meters in height and can live for more than a century (Abioye et al., 2014). The leaves of this tree are composed of several leaflets and have a digitated shape. They are consumed fresh or dried and are rich in proteins, vitamins (particularly vitamin C), minerals, fibers, and antioxidants (phenolic compounds, flavonoids, etc.) (Abiona et al., 2015; Habte et al., 2021). Baobab leaves are also recommended for combating gastroenteritis, fevers, and infectious diseases.

*C. olitorius* (Malvaceae), also known as jute or "corète potagère" in French and "kelen kelen" in the local Bafia language, is an annual herbaceous plant characterized by a slender stem. It is found in tropical regions of Africa (Cameroon, Nigeria, Ghana, Benin, etc.) and subtropical regions (Zimbabwe, Namibia, South Africa, etc.) due to its popularity as a leafy soup (Abdel-Razek et al., 2022). The leaves of this plant are rich in vitamins, minerals, and phenolic compounds. In addition to its culinary uses, these leaves are utilized in the treatment of typhoid and malaria fevers, dysentery, constipation, childhood malnutrition, and various pains (Osei-Owusu et al., 2023).

*T. cordifolia* (Malvaceae), known as Nkui in the Bamiléké language, is a woody plant characterized by a relatively short and remarkably ribbed trunk. It is also found in tropical regions of Africa (Cameroon, Gabon, Nigeria, Equatorial Guinea, Kenya, etc.). It can be perennial or annual, erect, and about 1 to 2 meters tall. The mature stem has a diameter of 1.5 to 2.5 cm and is generally greenish with star-shaped hairs, entire or lobed with frequently dentate margins (Saidou, 2012). The bark of this plant is particularly valued for its techno-functional potential due to its high fiber content (Grosser et al., 2018). Extracts from this bark are used to prepare a traditional and therapeutic dish (Nkui) highly prized in the Bamiléké region, and as an additive to improve dough volume in traditional baking.

It is therefore well established how important these various medicinal plants are for the nutrition and healthcare of African populations. However, one of the biggest problems that limits the actions of many therapists when recommending the use of these plants' organs in their various forms (powders, decoctions, infusions, etc.) and remains a major topic of reflection for many researchers is the lack of understanding of the effects of the constituents on the desired biological activities. Knowing that these activities are closely linked not only to the presence but also to the concentrations of each constituent (Cavalcante et al., 2022), this work seeks to highlight the influence of these two main factors (presence and contents) specific to nutrients (macro and micronutrients) and bioactive compounds (polyphenols, flavonoids, etc.) on the biological activities of the powders. One of the biological activities least associated with the three medicinal plants presented above is the translocation of the glucose storage protein type 4 (GLUT4).

The glucose transporter type 4 (GLUT4) plays a crucial role in glucose metabolism by facilitating its transport from the blood to muscle and adipose cells, where it is used to produce energy. When glucose enters the bloodstream after a meal, a precise concentration of insulin (ranging from 20 to 50 µunits/ml) is released by the pancreas and binds to its receptors on muscle and fat cells, triggering a cascade of cellular signals (Wang et al., 2020). This leads to 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) (Gannon et al., 2014). This reduction in glycemia due to insulin action begins almost immediately after food consumption. It is therefore evident that insulin is the primary trigger for GLUT4 translocation. However, several other bioactive molecules derived from medicinal plants have shown their ability to induce GLUT4 translocation (Sutandar et al., 2023). Nonetheless, very few of these molecules are related to the powders of the three medicinal plants mentioned above. Thus, the objective of this study was to determine the nutritional and bioactive composition of the powders from the leaves of *A. digitata*, *C. olitorius*, and the bark of *T. cordifolia*, and their effects on GLUT4 translocation activity.

1. **MATERIAL AND METHODS** 
   1. **Sampling, identification and powder production**

The dry leaves of *A. digitata* and the dry bark of *T. cordifolia* were purchased at the local market in Ngaoundéré, Adamaoua Region, while the leaves of *C. olitorius* were harvested early in the fields from vegetable gardens near Dibamba, Sanaga Maritime Department, Littoral Region. They were identified by the National Cameroonian Herbarium:

* *A. digitata*: The sample was identified by comparing it with material from collector Letouzey R number 7314 of specimen from Herbarium collection number. 7815 SRF/Cam
* *C. olitorius*: The sample was identified by comparing it with the herbarium material of Westphal collector 10077 from Herbarium collection specimen number 44871 SRF/Cam
* *T. cordifolia:* The sample was identified by comparing it with the Satabié material number 125 from the Herbarium specimen collection number 35712/HNC.

The powders were produced according to the requirements of therapists involved in the development of functional foods for the management of chronic diseases (Assiéné, Djeukeu, Assiéné, Mbida-Mbida, et al., 2024). Fresh leaves of *C. olitorius* were cleaned, washed, and cut into pieces approximately 5 mm in size. They were dried at 35°C for 10 hours in a Stockli dehydrator (Dorrex, France) and then ground into powders (particle size less than 500 µm) using a Philips blender (Model HR2058/00, 450W, 220–240 V~50–60 Hz, manufactured in China). The dry leaves of *A. digitata* and the dry bark of *T. cordifolia* were directly ground under the same conditions. The obtained powders were stored in an opaque glass container away from light at ambient temperature.

* 1. **Determination of proximate composition** 
     1. **Moisture content**

The moisture content of the powders was determined using the method of AOAC (1999) with slight modifications. Approximately 10 g of powder (M1) was placed in a pre-weighed capsule after being dried at 105 °C for 3 h and cooled in a desiccator. The whole (capsule and powder) was then dried in an oven (Model Memmert®, D 91,107 Schwabach, Germany) at 105 ± 2 °C for 18 to 24 h, followed by cooling to ambient temperature in a desiccator until it reached a constant weight of the powder (M2). The moisture content (MC) is calculated according to the formula:

* + 1. **Available carbohydrates**

The available carbohydrates were quantified using the method of Fischer & Stein (1961)**.** 0.5 g of the sample were precisely weighed into an Erlenmeyer flask equipped with a stopper and a delivery tube. Subsequently**,** 10 ml of 1.5 N sulfuric acid (H2SO4) was added. The mixture was heated to boiling for 45 min in a water bath and then allowed to cool to room temperature. Next, 10 ml of 70 % ethanol**,** 0.5 ml of zinc sulfate (2 g/100 ml), and 0.5 ml of potassium ferrocyanide (0.106 g/ml) were introduced. The resulting solution was filtered into a 50 ml volumetric flask and adjusted to the calibration mark. 0.25 ml of diluted glucose solution or glucose standard (0.25 to 1 mg**)** was pipetted into a test tube, followed by the addition of 0.5 ml of distilled water and 0.25 ml of DNS (3,5 Dinitrosalicylic acid). The entire solution was then heated in a water bath for 5 min, and the volume was subsequently adjusted to 5 ml with distilled water. After thorough mixing, optical densities were measured at 540 nm after cooling. The regression equa­tion derived from the glucose concentration range facilitated the necessary calculations, and the carbohydrate content is expressed as gram per 100 grams of dry matter (g/100 g DM).

* + 1. **Total protein and ash**

Total protein content was determined by the Kjeldahl method (AOAC, 1999). Total ash content was determined after incineration of the powders according to the protocol described by AOAC (1999).

* + 1. **Total lipids**

The total lipid content is determined using the hot extraction method in a Soxhlet apparatus, as described by Bourely (1982). The dried powders, obtained by drying at 105 °C, are placed in numbered filter papers, dried, and weighed. Oil extraction is carried out using hexane in the Soxhlet apparatus for a duration of 12 h. The oil content is calculated relative to the dry matter by the weight difference of the sachet before and after complete lipid extraction. The oil content (Oc) per 100 g of dry powder is given by the following formula:

M1: Weight of the filter paper sachet containing the powder before oil extraction

M: Weight of the filter paper sachet containing the powder after oil extraction

M2: Weight of the empty filter paper sachet

* + 1. **Crude fibers**

The crude fiber content of the powders was determined using the Weende method (Wolff, 1968). Approximately 20 g of powder (M) was introduced into a beaker containing 0.255 N sulfuric acid (H2SO4)**.** The mixture was brought to a boil for 30 min and then filtered. To the resulting residue, 0.313 N sodium hydroxide (NaOH) was added, and the entire mixture was boiled again for 30 min**.** After filtration, the residue was washed 3 times with hot distilled water and 2 times with acetone**.** The insoluble material obtained was dried at 105 ± 2 °C in an oven (Memmert®, D 91,107 Schwabach, Germany) for 8 h and weighed (M1). The resulting dry residue was incinerated at 550 °C for 3 h in a muffle furnace, and the obtained ashes were weighed (M2). The Crude fiber content (CF), expressed in gram per 100 g of dry matter (g/100 g DM), was calculated using the following formula:

Mc= Moisture content

* 1. **Determination of mineral contents**

The mineral extraction process for calcium (Ca), potassium (K), sodium (Na), copper (Cu), zinc (Zn), and iron (Fe) involves the following steps: Approximately 3 g of the powdered sample are subjected to dry ashing in a muffle furnace at a temperature of 500 °C for a duration of **6 h**. This process ensures com­plete combustion, leaving behind the mineral ash. The resulting ash is then diluted using a mixture of dilute hydrochloric acid (HCl) and nitric acid (HNO3)**.** This step prepares the sample for subsequent analysis. The analysis is performed using flame emission spectrometry (K, Na) and atomic absorption spectroscopy (Ca, Cu, Zn, Fe) (Jones & Venon, 1990). Specifically, an atomic absorption spectrophotometer (Model GBC, Sens AA, Dual, manufactured in the USA) is employed. This technique allows for the quantification of each mineral present in the sample. Phosphorus (P) is also extracted using the same dry ashing method. Subsequently, it is analyzed using the Murphy Riley reagent (Murphy & Riley, 1962) and read colorimetrically. The results are expressed in milligrams per 100 g of dry matter (mg/100 g DM) and grams per 100 g of dry matter (g/100 g DM) for trace element and macro-element, respectively**.**

* 1. **Preparation of 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 mins, followed by shaking for 15 mins. This process was repeated twice to maximize the extraction of secondary metabolites. The resulting solution was centrifuged for 10 mins at a speed of 13,400 revolutions per min, and the supernatant was collected, evaporated under reduced pressure, the freeze-drying.

* 1. **Determination of bioactive compound contents** 
     1. **Total polyphenol**

The determination of total polyphenols was performed by spectrophotometry, using the colorimetric method with the Folin-Ciocalteu reagent (Singleton et al., 1999). This assay is based on the quantification of the total concentration of hydroxyl groups present in the extract. The protocol used is based on that described by (Singleton & Rossi, 1965), with some modifications. Briefly, in glass hemolysis tubes, a volume of 500 µl of each extract was added, along with a mixture of 1 ml of Folin-Ciocalteu reagent diluted 10 times, and 800 µl of a 7.5% sodium carbonate solution (added 4 minutes later). The tubes were shaken and kept for 30 minutes. The absorbance was measured at 765 nm. A calibration curve was prepared in parallel under the same operating conditions using gallic acid at different concentrations (0 to 1000µg/ml).

* + 1. **Total flavonoids**

The determination of flavonoids was performed using a method based on the formation of a very stable complex between aluminum chloride and the oxygen atoms present on carbons 4 and 5 of flavonoids. The protocol used is based on that described by Zhishen et al. (1999) and Chun et al. (2003), with some modifications. In a glass hemolysis tube, 500 µl of extract, or standard, or distilled water for the control, were added to 200 µl of 5% NaNO2. After 5 minutes, 200 µl of 10% AlCl3 was added, and the mixture was vigorously stirred. After 6 minutes, a volume of 1000 µl of 1 M NaOH was added to the mixture. The absorbance was read immediately at 510 nm against the control. A methanolic solution of quercetin was prepared. Stock solutions were prepared from the mother solution at different concentrations ranging from 0 to 1000µg/ml to create the calibration curve.

* + 1. **Condensed tannins**

This assay was performed using the vanillin method combined with hydrochloric acid. This method relies on the reaction of vanillin with the terminal flavonoid group of condensed tannins, resulting in the formation of red complexes (Schofield et al., 2001; Makkar et al., 2007). This phenomenon is explained by the property of tannins to convert into red-colored anthocyanidols upon reaction with vanillin (Sun et al., 1998). The content of condensed tannins was determined using the vanillin method described by Julkunen-Tiitto (1985).A volume of 500 µl of each extract was added to 1500 µl of the 4% vanillin/methanol solution and then mixed vigorously. Next, a volume of 750 µl of concentrated hydrochloric acid (HCl) was added. The resulting mixture was allowed to react at room temperature for 20 minutes. The absorbance was measured at 550 nm against a blank. Different concentrations ranging from 0 to 1000µg/ml prepared from a stock solution of catechin were used to create the calibration curve.

* 1. **Evaluation of GLUT4 translocation activity**

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. Human insulin (Sigma Aldrich, Schnelldorf, Germany) was used as the reference hormone. The results obtained were expressed as a percentage of insulin (%Insulin).

* 1. **UPLC-UV-ESI-TOF-MS analysis of extract**

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 formic acid (0.1% in water; A) and acetonitrile (B), the B content was increased to 100% over 10 mins. The scan time for the MSe (centroid) method was set to 0.2 seconds. 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.

* 1. **Statistical analysis**

All analyses were conducted in triplicate (*n* = 3). Microsoft Excel 2016 software was employed for calculating means and standard de­viations. Statgraphic Centurion 15.2 software (StatPoint Technologies, Inc., Warrenton, Virginia, USA) was utilized for analysis of variance (one way and multiple way Anova), and means were separated using the Duncan multiple range test at a significance threshold of *P <* 0.05. SigmaPlot 11.0 software (Systat Software, Inc., 1735 Technology Drive, Suite 430, San Jose, CA 95,110, USA) was employed for graph plotting.

1. **RESULTS AND DISCUSSION**

* 1. **Moisture content**

Moisture content is a key factor that affects the biological stability of a food. This moisture must be removed or significantly reduced to extend the shelf life of the food. To this end, the moisture contents of the powders from the leaves of *A. digitata*, *C. olitorius*, and the bark of *T. cordifolia* were evaluated and presented in Table 1. It was observed that they varied significantly from 5.89 ± 0.02 to 12.50 ± 1.50 g/100g DM. These values indicate that, regardless of the powder studied, the obtained moisture contents do not favor the development of microorganisms that could degrade the hygienic quality of the powders. They are below 15 g/100g DM, the maximum value beyond which any biological degradation of food is possible (Rodríguez-Miranda et al., 2011). These results are similar to those (5.90 to 13.00 g/100g DM) reported by several authors on powders from various plant matrices (Assiéné et al., 2015 ; Assiéné, Djeukeu, Tizé, et al., 2024). Furthermore, moisture contents are also used to calculate the levels of many food constituents (proteins, carbohydrates, polyphenols, etc.) (Nielsen, 2009). This highlights the importance of measuring moisture in the evaluation of the proximate composition of the studied powders.

**Table 1**: Proximate composition (g/100g DM) and energy value of powdered leaves of *A. digitata, C. olitorius* and bark of *T. cordifolia*

|  |  |  |  |
| --- | --- | --- | --- |
| **Nutrients and energy** | ***A. digitata*** | ***C. olitorius*** | ***T. cordifolia*** |
| Moisture content | 8.90 ± 0.01b | 12.50 ± 1.50c | 5.89 ± 0.02a |
| Available carbohydrate | 37.72 ± 0.45a | 38.24 ± 0.20a | 58.02 ± 0.50b |
| Total proteins | 18.08 ± 0.5b | 21.60 ± 0.10c | 8.23 ± 0.30a |
| Total lipids | 4.15 ± 0.02c | 3.48 ± 0.05b | 2.10 ± 0.01a |
| Crude fibers | 19.05 ± 0.10b | 12.24 ± 0.50a | 18.98 ± 0.02b |
| Total ash | 10.78 ± 0.20b | 10.28 ± 0.35b | 4.16 ± 0.10a |
| Energie (Kcal/100g) | 257.75 ± 3.98a | 270.68 ± 1.65b | 283.90 ± 3.29c |
| Mn ± Sd: Mean ± Standard deviation; the affected means of different lowercase letters in superscript and on line show a significant difference at the probability threshold P ≤ 0.05 | | | |

* 1. **Nutritional composition**
     1. **Macronutrient content**

Table 1 presents the caloric nutrient contents of the powders from the leaves of *A. digitata, C. olitorius*, and the bark of *T. cordifolia*. These contents vary significantly from one powder to another, with values ranging from 37.72 ± 0.45 to 58.02 ± 0.50 g/100g DM for available sugars; 8.23 ± 0.30 to 21.60 ± 0.10 g/100g DM for total proteins; and 2.10 ± 0.01 to 4.15 ± 0.02 g/100g DM for total lipids. It is observed that the powders of *A. digitata*, *C. olitorius*, and *T. cordifolia* exhibit maximum lipid contents of 4.14 ± 0.02 g/100g DM, protein contents of 21.60 ± 0.10 g/100g DM, and sugar contents of 58.02 ± 0.50 g/100g DM, respectively. These lipid, protein, and sugar contents are higher than the 2.46 g/100g DM reported by Abioye et al. (2014), lower than the 27.32 g/100g DM noted by Sha’a et al. (2019), and nearly similar to the 63.11 g/100g DM reported by Ogbe & Affiku (2011) on the leaf powders of Moringa oleifera. The observed differences in caloric nutrient contents may be explained by multiple synthesis mechanisms occurring in each part of the plant. Indeed, numerous biological processes and cellular structures contribute to these synthesis mechanisms, including photosynthesis for sugars (in chloroplasts within the leaves), protein translation (in the nucleus of the plant cell), and lipogenesis (in the cytoplasm, involving the degradation of acetyl-CoA for fatty acid synthesis) (Moreau et al., 2017). These various biological processes are regulated by enzymes and can be influenced by numerous factors such as the environment, light, temperature, and nutrient availability. Additionally, post-harvest treatments (drying, grinding, etc.) applied to the plant parts may also influence the observed caloric nutrient contents (Fasuyi, 2005; Abioye et al., 2014), which ultimately impacts the energy value of each powder.

The energy values of the powders from *A. digitata*, *C. olitorius*, and *T. cordifolia* are 257.75 ± 3.98, 270.68 ± 1.65, and 283.90 ± 3.29 Kcal/100g, respectively (Table 1). These values are lower than the range of 331.07-359.87 Kcal/100g reported by Assiéné, Djeukeu, Assiéné, Mbida-Mbida, et al. (2024) for plant powders recommended in the fight against chronic diseases of dietary origin, such as type 2 diabetes. Indeed, the energy value of a powder is a key factor to consider before recommending it to a type 2 diabetic patient. Furthermore, the contribution of caloric nutrients to this energy value must be taken into account, prioritizing protein intake while reducing lipid and sugar intake (Markovic & Natoli, 2009). In this regard, the energy value of *C olitorius* powder (270.68 Kcal/100g) would be the most recommended for a diabetic patient who chooses to benefit from it as a dietary supplement. In this form, it is not only protein intake that must be considered; fibers and minerals are equally important for the diabetic patient.

Fibers are defined as carbohydrates that are not digested by human intestinal enzymes. They are essential in the process of stool evacuation along the intestinal lumen, and due to their satiating and chelating properties, they play a key role in regulating food intake. The crude fiber contents obtained from the powders of *A digitata*, *C olitorius*, and *T. cordifolia* are 19.05 ± 0.10, 12.24 ± 0.50, and 18.98 ± 0.02 g/100g dry matter (DM), respectively (Table 1). It is observed that there is no significant difference between the crude fiber contents of *A. digitata* powder (19.05 ± 0.10 g/100g DM) and *T. cordifolia* powder (18.98 ± 0.02 g/100g DM). However, these values are higher than the 11.96 g/100g DM reported by Abioye et al. (2014) for sun-dried *A. digitata* leaf powders. These differences can be explained by the previously mentioned reasons regarding caloric nutrients, as crude fibers (cellulose and hemicellulose) are primarily carbohydrates.

Total ash content indicates the amount of minerals present in the powders. The values obtained in this study vary significantly from one powder to another. They are 10.78 ± 0.20, 10.28 ± 0.35, and 4.16 ± 0.10 g/100g dry matter (DM) for the powders of *A. digitata*, C. olitorius, and *T. cordifolia*, respectively (Table 1). These values fall within the range of 7.25-26.79 g/100g DM reported by Patricia et al. (2014) for plant powders consumed in northern of Ivory Coast. They are also lower than the range of 6.30-7.23 g/100g DM reported by Ogbe & Affiku (2011) and (Raimi et al. (2014) for plant powders studied in Nigeria. However, it is essential to determine the quantities of each mineral of interest contained in these ashes before making any recommendations regarding the powder to a diabetic patient.

* + 1. **Mineral content**

Table 2 presents the trace element contents of the studied powders. No significant difference is observed between the zinc contents, with a maximum value of 98.71 ± 2.31 mg/100 g DM in *T. cordifolia* powder. In contrast, significant differences are noted in the copper and iron contents, with maximum values of 26.71 ± 1.27 mg/100 g DM in *C. olitorius* powder and 18.36 ± 1.29 mg/100 g DM in *A. digitata* powder, respectively. When comparing these values to those reported for powders regularly recommended by therapists to type 2 diabetic patients for their trace element contributions, such as the leaf powders of *M. oleifera* (2.46, 0.36, and 20.72 mg/100 g DM for zinc, copper, and iron, respectively) (Assiéné et al., 2015), it is observed that they are considerably higher. The same observation applies to other plant powders (6.01, 2.54, and 4.85 mg/100 g DM for zinc, copper, and iron, respectively), which are also significant in the management of type 2 diabetes (Assiéné, Djeukeu, Assiéné, Mbida-Mbida, et al., 2024).

Table 2 also shows that the macroelement contents (phosphorus, potassium, magnesium, calcium, sodium) of the studied powders vary significantly from one powder to another. However, only *C. olitorius* powder exhibits the highest contents of phosphorus (340.00 ± 2.10 mg/100 g DM), potassium (1957.41 ± 3.89 mg/100 g DM), magnesium (580.00 ± 1.45 mg/100 g DM), calcium (2200.00 ± 4.00 mg/100 g DM), and sodium (195.27 ± 1.00 mg/100 g DM). Compared to powders recommended for managing type 2 diabetes, these values are lower than 440 mg/100 g DM for phosphorus and 2410 mg/100 g DM for potassium, while being higher than 400 mg/100 g DM for magnesium, 1580 mg/100 g DM for calcium, and 150 mg/100 g DM for sodium reported by Assiéné et al. (2015) on *M. oleifera* leaf powders. These values are also lower than 88.39 mg/100 g DM for phosphorus, 639.68 mg/100 g DM for potassium, 24.30 mg/100 g DM for magnesium, 43.60 mg/100 g DM for sodium, and higher than 1120 mg/100 g DM for calcium reported by Assiéné, Djeukeu, Assiéné, Mbida-Mbida, et al. (2024) on other powders also recommended for managing chronic diseases of dietary origin, such as type 2 diabetes.

The observed differences in mineral contents, both for trace elements and macroelements, can be explained by several factors, including environmental conditions, topography, and seasons, which directly affect the absorption of minerals through plant roots from the soil. Additionally, post-harvest treatments applied to the harvested organs may also play a role (Nobosse et al., 2017). Indeed, the root hairs, which are tiny extensions of root cells, increase the root's surface area (greater absorption area), allowing for effective uptake of dissolved minerals in soil water. These minerals are then transported via water through the xylem vessels to the aerial parts of the plant, including stems and leaves. Many of these minerals found in the powders are involved in various metabolic reactions essential for plant development (Moreau et al., 2017). Furthermore, post-harvest treatments such as sieving may also influence the mineral contents of the powders.

**Table 2**: Mineral content of powdered leaves of *A. digitata, C. olitorius* and bark of *T. cordifolia* (mg/100g DM)

|  |  |  |  |
| --- | --- | --- | --- |
| **Mineral** | ***A. digitata*** | ***C. olitorius*** | ***T. cordifolia*** |
| **Trace element** | | | |
| Zinc (Zn) | 95.51 ± 2.34a | 94.44 ± 1.91a | 98.71 ± 2.31a |
| Copper (Cu) | 13.27 ± 0.93b | 26.71 ± 1.27c | 4.72 ± 0.09a |
| Iron (Fe) | 18.36 ± 1.29c | 14.05 ± 0.14b | 9.82 ± 0.50a |
| **Macro element** | | | |
| Phosphorus (P) | 121.85 ± 1.12b | 340.00 ± 2.10c | 96.60 ± 0.98a |
| Potassium (K) | 928.26 ± 2.89a | 1957.41 ± 3.89c | 967.50 ± 3.98b |
| Magnesium (Mg) | 63.18 ± 0.45b | 580.00 ± 1.45c | 58.32 ± 0.49a |
| Calcium (Ca) | 2088.00 ± 3.97b | 2200.00 ± 4.00c | 336.00 ± 2.00a |
| Sodium (Na) | 86.56 ± 0.78a | 195.27 ± 1.00c | 101.74 ± 1.05b |
| Mn ± Sd: Mean ± Standard deviation, the affected means of different lowercase letters in superscript and on line show a significant difference at the probability threshold P ≤ 0.05 | | | |

* 1. **Bioactive composition**

The contents of bioactive compounds in the studied powders are presented in Table 3. They vary significantly from 0.75 ± 0.10 to 2.92 ± 0.02 µg GAE/g dry matter (DM) for total polyphenols; from 0.58 ± 0.01 to 3.05 ± 0.00 µg CE/g DM for condensed tannins; and from 0.90 ± 0.02 to 4.90 ± 0.05 µg QE/g DM for total flavonoids. However, only *T. cordifolia* powder exhibits the highest contents of total polyphenols (2.92 ± 0.02 µg GAE/g DM), condensed tannins (3.05 ± 0.00 µg CE/g DM), and total flavonoids (4.90 ± 0.05 µg QE/g DM). These values are higher than those reported for polyphenols (0.113 µg CE/g DM) and flavonoids (0.016 µg QE/g DM) in *T. cordifolia* leaf powders collected in the city of Yaoundé (Foumane et al., 2022). They are also greater than those reported by Assiéné, Djeukeu, Tizé, et al. (2024) for polyphenols (0.271 µg GAE/100g DM), flavonoids (0.218 µg QE/100g DM), and tannins (0.016 µg CE/100g DM) in *Dacryodes edulis* bark powders.

The differences in the contents of bioactive compounds, which are primarily secondary metabolites, can be explained by the fact that they are synthesized by the plant in response to external stresses it encounters (temperature, human activity, rodents, etc.). Additionally, factors such as post-harvest treatments like grinding and sieving, physicochemical characteristics such as particle size and specific surface area of the granules, the extraction method of these compounds, and the type of solvent used can also account for the observed differences in the bioactive compound contents (Makkar et al., 2007). In light of these results, the powders from these three plant matrices exhibit immense bioactive potential that could be utilized in the fight against diabetes.

**Table 3**: Bioactive compounds of powdered leaves of *A. digitata, C. olitorius* and bark of *T. cordifolia*

|  |  |  |  |
| --- | --- | --- | --- |
| **Bioactive compounds** | ***A. digitata*** | ***C. olitorius*** | ***T. cordifolia*** |
| Total polyphenols (µg GAE/g DM) | 1.90 ± 0.10b | 0.75 ± 0.10a | 2.92 ± 0.02c |
| Condensed tanins (µg CE/g DM) | 0.58 ± 0.01a | 0.85 ± 0.01b | 3.05 ± 0.00c |
| Total flavonoids (µg QE/g DM) | 0.90 ± 0.02a | 2.3 ± 0.02b | 4.90 ± 0.05c |
| Mn ± Sd: Mean ± Standard deviation; the affected means of different lowercase letters in superscript and on line show a significant difference at the probability threshold P ≤ 0.05 | | | |

* 1. **Effects of nutritional composition and bioactive compounds of powders on GLUT4 translocation activity** 
     1. **GLUT4 translocation activity**

The translocation of glucose transporter type 4 (GLUT4) is an important biological activity in the regulation of blood glucose levels in individuals. It is activated by insulin secreted into the cell, which triggers a cascade of reactions that lead to the movement of GLUT4 storage vesicles to the cell membrane, allowing glucose to enter. Finding foods, or better yet, molecules that can mimic insulin or substitute for it to significantly reduce the insulin response is a considerable challenge. In this context, the GLUT4 translocation activity of powders from *A. digitata*, *C. olitorius*, and *T. cordifolia* has been evaluated and is presented in Fig. 1.

Fig. 1a shows the amount of GLUT4 translocated as a percentage of insulin (%Insulin) at different concentrations (50, 100, 200 µg/ml) of *A. digitata* powder extract over time (10, 20, 30 min). It is evident that both the duration of translocation activity and the concentration of extracts have highly significant effects (P = 0.0001, P = 0.0000, respectively) on the variations in the amount of GLUT4 translocated. However, the concentration of the extract has a greater effect (80.92%) compared to time (10.96%) on these variations. The values range from 35.65 ± 0.87 to 56.80 ± 0.20 %Insulin for a concentration of 50 µg/ml; 39.47 ± 0.13 to 66.77 ± 0.18 %Insulin for a concentration of 100 µg/ml; and 82.38 ± 0.32 to 90.61 ± 0.28 %Insulin for a concentration of 200 µg/ml. Notably, only the 200 µg/ml concentration shows the highest amount of GLUT4 translocated (90.61 ± 0.28 %Insulin) after 20 minutes of activity. This result indicates that the extracts from *A. digitata* powder at 200 µg/ml can activate GLUT4 translocation to 90.61% compared to insulin after 20 minutes of consumption.

Fig. 1b presents the amount of GLUT4 translocated as a percentage of insulin (%Insulin) at different concentrations (50, 100, 200 µg/ml) of *C. olitorius* powder extract over time (10, 20, 30 min). Similar to the *A. digitata* powder, both the duration of translocation activity and the concentration of extracts have highly significant effects (P = 0.0002, P = 0.0000, respectively) on the variations in GLUT4 translocation. Again, the concentration of the extract has a greater effect (75.53%) compared to time (13.19%). The values range from 38.96 ± 0.76 to 63.63 ± 0.14 %Insulin for a concentration of 50 µg/ml; 40.25 ± 0.74 to 68.65 ± 0.17 %Insulin for a concentration of 100 µg/ml; and 82.44 ± 0.13 to 92.53 ± 0.01 %Insulin for a concentration of 200 µg/ml. The extracts from *C. olitorius* powder at 200 µg/ml can also activate GLUT4 translocation to 92.52% compared to insulin after 20 minutes of consumption.

Fig. 1c illustrates the amount of GLUT4 translocated as a percentage of insulin (%Insulin) at different concentrations (50, 100, 200 µg/ml) of *T. cordifolia* powder extract over time (10, 20, 30 min). Both the duration of activity and the concentration of extracts have highly significant effects (P = 0.0000; P = 0.0000, respectively) on the variations in GLUT4 translocation. However, these effects are toxic, with these two factors accounting for 40.06% and 57.34%, respectively.

 

**(b)** 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%

1. The time effect: Sum of squares = **10.96%, P = 0.0001**; Extract concentration effect: Sum of squares **= 80.92%, P = 0.0000**; Residual: Sum of squares = 8.11%



**(c)** The time effect: Sum of squares **= 40.06%,** **P = 0.0000**; Extract concentration effect: Sum of squares **= 57.34%,** **P = 0.0000**; Residual: Sum of squares = 2.60%

**Fig. 1.** GLUT4 translocation activity of extracts from powdered leaves of *Adansonia digitata* (a), *Corchorus olitorius* (b) and Bark of *Triumfetta cordifolia* (c) at different concentrations (50, 100, 200 µg/ml) 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.)

* + 1. **Effects of nutritional composition and bioactive compounds**

Regardless of the studied powder, the GLUT4 translocation activity and its variations are certainly due to the nutritional and bioactive composition of the powders. To this end, a correlation test between the constituents of the powders and the GLUT4 translocation activity at 200 µg/ml was conducted and is presented in Table 4. It shows, on one hand, that between GLUT4-10, GLUT4-20, GLUT4-30 and available sugars, there are high significant negative correlations (r = -0.997, P = 0.044); (r = -0.998, P = 0.039); (r = -0.997, P = 0.043) and with calcium, there are high significant positive correlations (r = 0.998, P = 0.022); (r = 0.999, P = 0.027); (r = 0.998, P = 0.024), respectively. On the other hand, there are also high non-significant negative correlations between GLUT4-10, GLUT4-20, GLUT4-30 and bioactive compounds, particularly total polyphenols (r = -0.857, P = 0.343); (r = -0.853, P = 0.348); (r = -0.856, P = 0.345); condensed tannins (r = -0.993, P = 0.075); (r = -0.994, P = 0.070); (r = -0.993, P = 0.073); and total flavonoids (r = -0.932, P = 0.235); (r = -0.935, P = 0.230); (r = -0.933, P = 0.234), respectively.

These results indicate that as GLUT4 translocation activity increases, the levels of available sugars and bioactive compounds decrease. This increase in GLUT4 is proportional to the levels of calcium. This suggests that bioactive compounds play a role in the biological mechanisms that trigger GLUT4 translocation to reduce the amount of sugars provided. However, this GLUT4 translocation activity and the reduction in sugar content depend on the amount of available calcium. Due to their significance, the levels of available sugars and calcium, unlike those of bioactive compounds, are two key factors that would condition GLUT4 activity.

When comparing these results to the literature, it is evident that, unlike many other minerals, calcium plays a crucial role in the mechanisms of GLUT4 translocation. Indeed, calcium is essential for cellular signaling, the activation of protein kinases, and the mobilization of GLUT4 reserves (Dombrowski, 2001). When insulin binds to its receptor on the membrane, it activates a cascade of intracellular signaling that includes an increase in intracellular calcium levels. This increase in calcium is essential for GLUT4 translocation. Intracellular calcium activates certain protein kinases, such as protein kinase C (PKC) and protein kinase B (Akt), which are also involved in GLUT4 translocation. These kinases phosphorylate specific proteins that facilitate the movement of GLUT4 to the plasma membrane. The intracellular reserves of GLUT4 stored in vesicles require calcium for their mobilization. These vesicles fuse with the plasma membrane, allowing for increased glucose absorption (Li et al., 2014). From the above, it is clear that a significant decrease in calcium can disrupt the entire GLUT4 translocation mechanisms and significantly affect the entry of sugar, particularly glucose, into cells. This important role of calcium in GLUT4 translocation highlights its essential nature in the diet of diabetic patients.

Although the bioactive compounds (total polyphenols, condensed tannins, total flavonoids) did not show any significance under the study conditions, one cannot overlook the fact that their content or even their presence contributes to the movement of GLUT4 storage vesicles to the plasma membrane. This can only be attributed to the action of specific bioactive molecules contained in the powders. To this end, a determination of the bioactive compound profiles of the extracts from each powder was conducted and is presented in Figs. 2, 3, and 4.

Figs. 2 and 3 each present a chromatogram coupled with a mass spectrum that highlight the presence of several chemical compounds contained in the extracts from *A. digitata* and *C. olitorius* powders, respectively. When comparing the identified chemical compounds with literature data, it is observed that in the *A. digitata* powder (Fig. 1), the following flavonoids are known for their GLUT4 translocation activities: Procyanidin B1, Procyanidin C1 and C2; kaempferol-3-O-rhamnosylrutinoside, rutin, isoquercetin, and quercetin (Yamashita et al., 2016; Kadan et al., 2021; Sutandar et al., 2023). Meanwhile, in the *C. olitorius* powder (Fig. 2), isoquercetin and a quercetin glycoside (quercetin 3-O-(6˝-acetyl-glucoside)) are also found (Jiang et al., 2019; Yamashita et al., 2024). These results may explain the high GLUT4 translocation activities of the extracts from *A. digitata* powder (90.61% Insulin) and C. olitorius powder (92.52% Insulin) (Figs. 1a and 1b).

Fig. 4 presents a chromatogram coupled with a mass spectrum that highlights the presence of several chemical compounds contained in the extracts from *T. cordifolia* powders. Several chemical compounds are identified that do not belong to the polyphenol family, nor to flavonoids or condensed tannins, including D-gluconic acid, quinic acid, (+)-lyoniresinol 9’-O-glucoside, cryptostictic acid, fatty acids, and triterpenes. These organic compounds have no GLUT4 translocation activity when compared to literature data. However, their high content in the powders could have a toxic effect on the amount of GLUT4 translocated, potentially explaining the observed results on GLUT4 translocation from the extracts of *T. cordifolia* powder (Fig. 1c).

Table 4 : Main significant and non-significant high correlations between certain nutrients, bioactive compounds, and GLUT4 translocation activities

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  | Available carbohydrate | Calcium | Total polyphenol | Condensed tannins | Total flavonoids |
| Available carbohydrate | 1 | **- 0.999 (0.007)** | / | / | / |
| GLUT4-101 | **- 0.997 (0.044)** | **0.998 (0.022)** | -0.857 (0.343) | -0.993 (0.075) | -0.932 (0.235) |
| GLUT4-202 | **- 0.998 (0.039)** | **0.999 (0.027)** | -0.853 (0.348) | -0.994 (0.070) | -0.935 (0.230) |
| GLUT4-303 | **- 0.997 (0.043)** | **0.998 (0.024)** | -0.856 (0.345) | -0.993 (0.073) | -0.933 (0.234) |
| Correlation coefficient (probability threshold), Probability values below 0.05 indicate correlation coefficients (in bold) that are significantly different from 0 at a 95.0% confidence level. 1 GLUT4 translocation after 10 minutes of activity on average; 2 GLUT4 translocation after 20 minutes of activity on average; 3 GLUT4 translocation after 30 minutes of activity on average | | | | | |



Fig. 2. UPLC/ESI-TOF-MS chromatogram of *Adansonia digitata* leaf extracts



Fig. 3. UPLC/ESI-TOF-MS chromatogram of *Corchorus olitorius* leaf extracts



Fig. 4. UPLC/ESI-TOF-MS chromatogram of *Triumfetta cordifolia* bark extracts

1. **CONCLUSION**

This study aimed to determine the nutritional and bioactive composition of leaf powders from *A. digitata, C. olitorius*, and the bark of *T. cordifolia*, as well as their effects on GLUT4 translocation activity. The results indicate that the powders from these plants have significant nutritional potential, with high levels of essential nutrients, including proteins, trace elements (iron, zinc, copper), macroelements (calcium, magnesium), and crude fiber, known for their satiety-inducing and dietary regulating properties. In this regard, the powders of *C. olitorius* followed by A. digitata exhibit better nutritional potentials that can be recommended for type 2 diabetic patients. The bioactive potential, relative to the levels of bioactive compounds (total polyphenols, condensed tannins, total flavonoids), is higher in the powder of *T. cordifolia*. However, it is the only powder that exhibits toxic effects on GLUT4 translocation activity. In contrast, the powders of *C. olitorius* and *A. digitata* demonstrate significant GLUT4 translocation activity, likely triggered by numerous bioactive compounds from the flavonoid family (kaempferol-3-O-rhamnosylrutinoside, isoquercetin, quercetin, etc.), in which calcium levels play a crucial role. Nevertheless, a more in-depth study, particularly a bio-guided fractionation, will follow this research to more precisely identify the bioactive molecules responsible for the observed GLUT4 translocation activity. However, these results can nonetheless help guide type 2 diabetic patients toward the consumption of *C olitorius* powder, as it presents a greater nutritional and bioactive potential.

**DISCLAIMER (ARTIFICIAL INTELLIGENCE)**

Authors hereby declare that no generative AI technologies such as large language Models (ChatGPT, Copilot, etc.) and text to image generators have been used during the writing or editing of this manuscript

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