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Antihyperglycemic activity of crude extract and isolation of phenolic compounds with antioxidant activity from *Moringa oleifera* Lam. leaves grown in Southern Brazil

Tatiane Luiza C. Oldoni [a,*], Nathalie Merlin [a], Thariane Carvalho Bicas [a], Anaclara Prasniewski [b], Solange Teresinha Carpes [a], Jociani Ascari [b], Severino Matias de Alencar [c], Adna Prado Massarioli [c], Margarete Dulce Bagatini [d], Rafael Morales [e], Gustavo Thomé [a]

[a] Department of Chemistry, Federal Technological University of Paraná (UTFPR), Pato Branco, PR 85503-390, Brazil
[b] Department of Biology, Federal Technological University of Paraná (UTFPR), Santa Helena, PR 85892-000, Brazil
[c] Department of Agri-Food Industry, Food and Nutrition, "Luiz de Queiroz" College of Agriculture, University of Sao Paulo (USP), P.O. Box. 9, 13418-900 Piracicaba, SP, Brazil
[d] Coordenação Acadêmica, Universidade Federal da Fronteira Sul, Chapecó, SC, Brazil
[e] Empresa de Pesquisa Agropecuária e Extensão Rural de Santa Catarina (Epagri), 88318-112 Itajaí, SC, Brazil

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ABSTRACT

The antihyperglicemic activity of crude extract from *Moringa oleifera* leaves and isolation of phenolic compounds with antioxidant activity using bioguided assay were employed by the first time in leaves cultivated in Brazil. The hydroalcoholic extract (HE) was produced by using ethanol:water (80:20 v/v) and purified by solid–liquid procedure using solvents in ascending order of polarity. The ethyl acetate fraction (Fr-EtOAc) presented high antioxidant potential and it was purified using chromatographic techniques rendering isolated compounds that were identified from the spectral data. The HE extract (500 mg kg^{-1}) was adimistrated in diabetic rats induced by streptozotocin and chemical markers and lipid peroxidation in liver and kidney were evaluated. The Fr-EtOAc showed high antioxidant potential by FRAP reduction method (1678 μmol Fe^{2+} g^{-1}), DPPH and ABTS scavenging methods (526.7 and 671.5 μmol TEAC g^{-1} respectively) and ORAC assay (3560.6 μmol TEAC g^{-1}). Therefore, the Fr-EtOAc was purified and yielded three bioactive subfractions (S-12, S-13 abd S-15) that were rechromatoghaphed in HPLC-SemiPrep. After that, two main bioactive glycosylated flavonoids (isoquercitrin and astragalin) and phenolic acid (3-O-caffeoylquinic acid) were obtained. Additionally, the HE extract provided protection against oxidative damage in liver and kidney of diabetic rats ameliorating endogenous antioxidant defenses by increase catalase (CAT), glutathione S-transferase (GST) and non-protein thiol groups (NPSH) levels as well as decreased the lipid peroxidation in these tissues. Our results indicate that three phenolic compounds with high antioxidant activity were isolated and, the chemical composition of HE crude extract, rich in flavonoids glycosylated could be intimately related to antihyperglycemic action. So, it is possible to suggest that these compounds may be used as chemical biomarkers for this plant in Brazil, ensuring quality and supporting the use of aerial parts in tradicional medicine.

1. Introduction

Moringa oleifera Lam. (Moringaceae) is a tree widely cultivated in South of Asia for its biological and nutritional value (Nambiar & Seshadri, 2001). In Brazil, it is popularly known as "white acacia" and "moringueiro" (Amaya et al., 1992) and the tree has adapted well to climate of warm regions of Brazil. Nowadays, it can be found in the Brazilian northeastern semiarid due to application of seeds in water treatment for domestic use (Pereira et al., 2018). The main traditional uses of genus *Moringa* are compiled in a recent review of Rani et al (2018) and leaves of *M oleifera* are used to treat diabetes, hypertension, skin diseases, colitis, anemia, sign of aging, anxiety (Abe & Ohtani, 2013; Silver, 2017). In addition, the extracts present antibacterial and antimalarial uses (Parrotta, 1993).

* Corresponding author.at: Department of Chemistry, Federal Technological University of Paraná (UTFPR), Pato Branco, PR 85503-390, Brazil.
E-mail address: tatianeoldoni@utfpr.edu.br (T.L.C. Oldoni).

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The medicinal properties can be related to the complex chemical composition which is rich in phytochemicals that present a wide range of biological activities (Stohs & Hartman, 2015) as antioxidant, anti-inflamatory, antilipidemic (Omodanisi et al., 2017), sedative and anti-diabetic potential (Bakre et al., 2013; Zainab et al., 2020). The phenolic compounds, mainly phenolic acids and complex flavonoids derivative of quercetin, isorhamnetin and kaempferol (Castro-López et al., 2017; Nouman et al., 2016; Oldoni et al., 2019a, Oldoni et al., 2019b; Ramabulana et al., 2016; Rodríguez-Pérez et al., 2015; Shervington et al., 2018) were identified as biological compounds in the leaves.

Studies have demonstrated the antihyperglycemic potential of ethanolic and aqueous extracts of *M. oleifera* leaves. Diabetes mellitus is a chronic metabolic disorder of carbohydrate metabolism that is originated from abnormal insulin secretion or action, which results in hyperglycemia that can affect many organs and cause disturbances in the body imune system (Lin et al., 2018). According to Tende et al (2011) and Jaiswal et al (2009) the administration of ethanolic and aqueous extracts of *M. oleifera* leaves can reduce insulin resistance with decreased blood glucose levels and this effect can be related to presence of terpenoids and phenolic compounds.

A decrease in antioxidant defenses and an increase of reactive oxygen species (ROS) can be produced continuously after long-term exposure to hyperglycemic condition and, this state could decrease *in vivo* antioxidants levels resulting in diabetes. Thus, products rich in antioxidants may play a key role in treatment of diabetes (Zheng et al., 2017). In addition, phenolic compounds target pancreatic β-cells and act as insulin secretagogues to maintain glucose homeostasis upon hyperglycemia (Ghani, 2020).

Polyphenols are secondary metabolites produced by plants and its main functions are to modulate enzymes, cell receptors as well as to provide protection against parasites, UV radiation and virus. In addition, these class of compounds are responsible for the biological activities as antioxidant and hypoglycemic (Manach et al., 2005; Omodanisi et al., 2017) mainly because they complement the antioxidant system endogenous defense. The phenolic groups found in natural products are phenolic acids, lignans, stilbenes and, mainly flavonoids, that present a chemical strucuture able to capture free radicals, superoxide anions, hidroxil radicals by electron transfer (Williams et al., 2004) and, in addition, can promote an increase in activities of some antioxidants enzymes. The biological properties of phenolic compounds are related to conjugation, glycosylation or methylation and the hydrophilicity of these compounds (Romanos-Nanclares et al., 2020; Williams et al., 2004).

The antioxidant potential of *M oleifera* extracts can be intimately related to potent action of glycosilated flavonoids isoquercitrin (1) and astragalin (2) and phenolic acid chlorogenic (3) isolated in this study. These molecules have therapeutic action and have been shown increasing attention due to health benefits and biological activities which increase the protective effects regarding different diseases. For Ahangarpour et al (2019) the structure of chlorogenic acid enables its activity similar to metformin in potentiating insulin action while Rey et al (2019) suggest that astragalin can contribute to glucose homeoastase through the influx of calcium into the K^+ATP, L-VDCC channels, mobilizing calcium from intracellular reserves and activating the PKC and PKA kinase proteins, leading to insulin secretion.

The isolation of bioactive compounds by using the bioguided fractionation technique is an important tool to separate components which present some biological activity and the main advantage is the rationalization of the process used to isolate biologically bioactive substances from complex natural extracts (Chen et al., 2017; Dikpınar et al., 2018; Oldoni et al., 2016; Ortiz et al., 2019). In studies that use isolation at random it is not possible to ensure that the compounds responsible for the activity of interest were, in fact, isolated. Thus the isolated compounds by using bioguided isolation can be considered chemical markers for the chemical evaluation and quality control of plants (Li et al., 2013).

Although few studies have been carried out with *M. oleifera* leaves cultivated in Brazil, it is possible to suggest that this plant have phenolic compounds that present functional and biological activities. Therefore, the aim of the present study was to evaluate the antioxidant and anti-hyperglycemic activity of crude extract, and isolate phenolic compounds using the bioguided-assay technique based on antioxidant assays from fractions and subfractions.

2. Materials and methods

2.1. Chemicals

The sílica gel (70–230 mesh) and thin layer chromatography (TLC) DC-Fertigfolien Alugram Xtra SIL UV254 were purchased from Merck & Co., Inc. Analytical reagent-grade hexane (Hex), dichloromethane (CH_2Cl_2), ethyl acetate (EtOAc), acetone (Ace), ethanol (EtOH), 2,2-difenil-1-picril-hidrazil (DPPH), 2,2′-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), (±)-6-Hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (Trolox), 2,2′-azo-bis(2-amidinopropane) dihydrochloride (AAPH); 2,4,6-Tri(2-pyridyl)-s-triazine (TPTZ), potassium persulfate, potassium phosphate buffer and fluorescein were obtained from Sigma Chemical, SP, Brazil. The reagents acetic acid and all the solvents HPLC grade (J. T. Baker, Phillipsburg, NJ, USA); sodium carbonate (Na_2CO_3) and Folin-Ciocalteu reagent (Dinâmica, Diadema, SP, Brazil).

2.2. Plant material

The leaves of *M. oleifera* were collected in Itajaí city, Santa Catarina state, Southern Brazil (geographical coordinates: 26° 57′ 16.2″ S and 48° 45′ 55.0″ W) at March of 2015. A voucher specimen (HPB 483) was authenticated by a plant taxonomist, Giovana Faneco Pereira, and deposited in the Herbarium of Federal Technological University of Paraná (Pato Branco, Paraná, Brazil). After collected, leaves were oven-dried (at 40–45 °C), ground, weighed and stored under refrigeration.

2.3. Extraction and isolation of phenolic compounds

Plant material (1 kg) was extracted with 2 L of Ethanol:water (80:20, v/v) under ocasional stirring, for 48 h at ambient conditions of temperature (22–25 °C) and light. The extract was filtered and stored under refrigeration and this procedure was repeated three times to guarantee the complete extraction of compounds. After mixing the three filtrates, they were concentrated using a rotary evaporator, at 40 °C, lyophilized and resulted in Hydroalcoholic Extract (HE) that was purified and used for all described assays. The HE (325 g) was fractioned by solid–liquid extraction using hexane (Fr-Hex; 45.7 g), dichloromethane (Fr-CH_2Cl_2; 8.60 g), ethyl acetate (Fr-EtOAc; 1.71 g), acetone (Fr-Ace; 5.18 g), ethanol (Fr-EtOH; 75.3 g) and ethanol:water (50:50, v/v) (Fr-Hydro; 104 g). At regular intervals of 24, 48 and 72 h the solvents were replaced and at the end of process the organic solvents were evaporated and residual water was lyophilized (Fr-Hydro). The active Fr-EtOAc (801 mg) was purified in an open Column Chromatography on normal phase sílica gel and eluted by using Hex, EtOAc, EtOH and water to afford 61 subfractions. The obtained subfractions were applied in TLC and the subfractions that showed similar color (under natural and UV light) and Rf were regrouped into 18 subfractions. All the subfractions were evaluated for their antioxidant activity and chemical profile using HPLC. The assays were carried out on a Varian 920-LC using an Agilent Microsorb-MV 100 C18 column (250 × 4.6 mm, 5 μm particle size). A mixture of water: acetic acid (98:2, v/v) (solvent A) and acetonitrile:water:acetic acid (40:58:2, v/v) (solvent B) was used in a gradient mode as mobile phase. The flow rate was 1.0 mL min^{-1} in gradient mode, starting with 5% solvent B to 20% B in 2 min, 25% B in 15 min, 85% B in 25 min, 85% B in 30 min, 95% B in 32 min, 95% B in 33 min and 5% B in 36 min. The

temperature of column was maintained at 30 °C. The retention time (RT) and absorption profile in ultraviolet region were compared with authentic standards for identification of signals (Oldoni et al., 2019a, Oldoni et al., 2019b). The most bioactive subfractions S-12, S-13 and S-15 were purified by semipreparative HPLC using a Shimadzu Shim-pack PREP-ODS (H) (250 × 20 mm) column eluted in a gradient mode with water (solvent A) and a mixture of acetonitrile and water (40:60, v/v) (solvent B) with a flow rate of 7 mL min^{-1} at 30 °C using a photo-diode-array(PDA) detector.

2.4. Nuclear magnetic resonance (NMR)

The NMR spectra, obtained in CD_3OD using $SiMe_4$ as internal standard, were recorded in a Brucker DPX 500 MHz spectrometer, operating at 500 MHz for ^1H and 125 MHz for ^{13}C. The chemical shifts are expressed in δ (parts per million) and the coupling constants (J) in Hz. NMR assignments were made by a combination of 1D and 2D techniques and by comparison with those made for previously described compounds, where appropriate.

Isoquercitrin (**1**): UVmax (EtOH): 254, 354 (sh); MS *m/z* (rel. int.): 465.1024 $(M+H)^+$, 303.0498 (100), 304.0530 (17.2), 85.0278 (3), $C_{21}H_{20}O_{12}$. ^1H NMR (500 MHz, CD_3OD): δ ppm 7.71 (d, 1H, $J = 2.0$ Hz, H-2′), 7.58 (dd, 1H, $J = 2.0$, 8.5 Hz, H-6′), 6.86 (d, 1H, $J = 8.5$ Hz, H-5′), 6.34 (d, 1H, $J = 15$ Hz, H-8), 6.16 (d, 1H, $J = 1.5$ Hz, H-6), 5.21 (d, 1H, $J = 7.5$ Hz, H-1″), 3.22 (m, 1H, H-5″), 3.35–3.37 (m, 1H, H-4″), 3.42 (m, 1H, H-3″), 3.48 (m, 1H, H-2″), 3.71 (dd, 1H, $J = 2.5$, 11.5 Hz,H-6a"), 3.58 (dd, 1H, $J = 5.5$, 12.0 Hz, H-6b"). ^{13}C NMR (125 MHz, CD_3OD): δ ppm 177.4 (C-4), 164.51 (C-7), 161.7 (C-5), 157.5 (C-2 and C-9), 148.7 (C-3′), 144.9 (C-4′), 134.5 (C-3), 122.0 (C-6′), 116.1 (C-2′), 114.5 (C-5′), 104.04 (C-10 and C-1″), 99.8 (C-6), 94.0 (C-8), 77.3 (C-3″ e C-5″), 74.8 (C-2″), 70.6 (C-4″), 61.4 (C-6a“ and C-6b") (Kazuma et al., 2003).

Astragalin (**2**): UVmax (EtOH): 263, 346 (sh); MS *m/z* (rel. int.): 449.1076 $(M+H)^+$, 287.0549 (100), $C_{21}H_{20}O_{11}$. ^1H NMR (500 MHz, CD_3OD): δ ppm 8.05 (d, 2H, $J = 9.0$ Hz, H-2′, H-6′), 6.88 (d, 2H, $J = 9.0$ Hz, H-3′, H-5′), 6.34 (d,1H, $J = 2.0$ Hz, H-8), 6.16 (d, 1H, $J = 2.0$ Hz, H-6), 5.21 (d, 1H, $J = 7.5$ Hz, H-1″), 3.41–3.44 (m, 2H, H-2″, H-3″), 3.30–3.35 (m, 1H, H-4″), 3.20 (m, 1H, H-5″), 3.53 (dd, 1H, $J = 5.5$, 12 Hz, H6b"), 3.69 (dd, 1H, $J = 2.0$, 12 Hz, H6a"). ^{13}C NMR (125 MHz, CD_3OD): δ ppm 179.47 (C-4), 163.14 (C-7), 161.74 (C-5, C-4′), 156.01 (C-9), 158.80 (C-2), 135.55 (C-3), 132.39 (C-2′, C-6′), 121.98 (C-1′), 116.23 (C-3′, C-5′), 105.38 (C-10), 104.41 (C-1″), 100.62 (C-6), 95.30 (C-8), 78.55 (C-5″), 78.22 (C-3″), 75.88 (C-2″), 71.50 (C-4″), 62.77 (C-6″ a, b) (Kazuma et al., 2003; Wei et al., 2011)

3-O-caffeoylquinic acid (**3**): UVmax (EtOH): 324, (sh); MS *m/z* (rel. int.): 353.0836 $(M-H)^-$, 191.0530 (100), 179.0321 (88), 173.0427 (32), 135.0428 (20), $C_{16}H_{18}O_9$. ^1H NMR (500 MHz, CD_3OD): δ ppm 7.58 (d, 1H, $J = 16.0$ Hz, H-8′), 7.05 (d, 1H, $J = 2.0$ Hz, H-2′), 6.94 (dd, 1H, $J = 2.0$, 8.0 Hz, H-6′), 6.76 (d, 1H, $J = 8.0$ Hz, H-5′), 6.31 (d, 1H, $J = 16.0$ Hz, H-7′), 5.41 (quintet, 1H, $J = 4.0$ Hz, H3), 3.98 (quartet, 1H, $J = 5.4$ Hz, H5), 3.81 (m, 1H, H-4), 2.03–2.10 (m, 2H, H-6), 1.89–2.00 (m, 2H, H-2). ^{13}C NMR(125 MHz, CD_3OD): δ ppm 180.0 (COO), 167.29 (C-9′), 148.25 (C-4′), 145.67 (C-7′), 145.42 (C-3′), 126.44 (C-1′), 121.48 (C-6′), 115.12 (C-5′), 114.23 (C-8′), 113.63 (C-2′), 74.46 (C-1), 71.81 (C-4), 70.07 (C-3), 68.87 (C-5), 37.38 (C-6), 36.08 (C-2) (Chan et al., 2009).

2.5. Antioxidant activity in vitro

2.5.1. Reducing capacity of the Folin-Ciocalteu reagent (F-C assay)

The methodology suggested by Singleton et al (1999) was used to quantify phenolic compounds. In test tubes reagents were added in order: 0.5 mL of HE (300 mg/L), fractions or subfractions (500 mg/L); 2.5 mL of Folin-Ciocalteau reagent (10%); and 2.0 mL of sodium carbonate (Na_2CO_3 4%). After 2 h at ambient temperature and in the dark, the absorbances were measured on a spectrophotometer (model UV–VIS Lambda 25, Perkin Elmer) at 740 nm. The analytical blank was performed using 0.5 mL of ultra-pure water with Folin-Ciocalteau and sodium carbonate reagents. The results were expressed as gallic acid equivalents (GAE, mg/g), based on the calibration curve plotted using absorbance versus concentration (5 to 100 mg/L) of gallic acid.

2.5.2. Ferric reducing antioxidant power (FRAP)

To obtain the FRAP reagent were mixed 25 mL of acetate buffer (300 mmol/L, pH 3.6); 2.5 mL of TPTZ (10 mmol/L in 40 mmol L^{-1} HCl) and 2.5 mL of $FeCl_3$ (20 mmol/L in aqueous solution). In test tubes reagents were added: 90 μL of HE and subfractions at 300 mg/L, while fractions were evaluated at 1000 and 500 mg/L, for Fr-CH_2Cl_2 and others respectively; then 270 μL of water and 2.7 mL of FRAP reagent (Benzie & Strain, 1996). The test tubes were kept in water bath (37 °C) for 30 min. After reaction time the absorbances of samples and solutions of calibration curve (Fe_2SO_4) were read at 593 nm. For quantification pourposes was plotted a calibration curve relating absorbance versus concentration (from 100 to 1500 μmol/L) and results were expressed as μmol Fe^{2+}/g.

2.5.3. 2,2-diphenyl-1-picrylhydrazyl ($DPPH^•$) assay

In test tubes reagents were mixed in order: 0.5 mL of HE and subfractions at 100 mg/L and fractions (1000, 500 and 100 mg/L, for Fr-CH_2Cl_2, Fr-Hex and others, respectively); 3.0 mL of EtOH:H_2O (80:20 v/v); and 0.3 mL DPPH radical (0.5 mmol/L) in ethanolic solution. After 45 min at room temperature the absorbances were measured at 517 nm using Trolox (15 to 100 μmol/L) as reference. The results were expressed as μmol of Trolox equivalent antioxidant capacity (TEAC) g^{-1} of extract, fraction or subfractions (Brand-Williams et al., 1995).

2.5.4. 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) ($ABTS^{•+}$) assay

To obtain the ABTS cation radical was mixed a solution stock of ABTS (7 mmol/L) with 140 mmol/L potassium persulfate and, after 16 h in the dark, the solution containing $ABTS^{•+}$ was diluted by using ethanol until absorbance value of approximately 0.700 at 734 nm. In test tubes reagents were added 3.0 mL of the $ABTS^{•+}$ solution freshly prepared; 30 μL of HE and subfraction at 300 mg/L and fractions (3000, 1000 and 500 mg/L, for Fr-Hex, Fr-CH_2Cl_2 and others, respectively). The reference Trolox was used for quantificantion pourposes in a range of 100 to 2000 μmol/L. After 6 min of reaction, at ambient temperature and in the dark, the absorbances were read spectrophotometrically at 734 nm. The results were expressed as μmol TEAC/g of extract, fraction or subfractions (Re et al., 1999).

2.5.5. Oxygen radical absorbance capacity (ORAC)

In test tubes reagents were added 30 μL of HE and fractions at 50 mg/L, subfractions at 300 mg/L; 60 μL of fluorescein solution at 508 nmol/L; and 110 μL of 76 mmol/L 2,2′-azobis(2-amidinopropane) dihydrochloride (AAPH) solution. The solutions of fluorescein and AAPH were prepared in 75 mmol/L phosphate buffer (pH 7.5) (Chisté et al., 2011). For quantification pourposes a calibration curve was plotted with Trolox reference. The fluorescence signal was monitored every minute, during 2 h at 37 °C, with a microplate reader (Molecular Devices SpectraMax M3). The emission and excitation wavelengths were 528 nm and 485 nm.

2.6. Biochemical parameters and cell viability

2.6.1. Isolation of peripheral blood mononuclear cells (PBMCs)

Peripheral blood mononuclear cells were isolated from fresh blood from healthy volunteers (Ethics Committee No. 822.782) using Ficoll-Paque PLUS (GE Healthcare Bio-Science, Darmstadt, Germany) (Boyum, 1976). Blood samples were collected in tubes containing EDTA, and subsequently, in 1 to 2 h, they were diluted in PBS (phosphate buffered saline) and separated by Ficoll-Paque PLUS in the 400 g centrifugation for 40 min. Briefly, the PBMCs were carefully collected from the supernatant and the pellet was resuspended in 1 mL RPMI

medium (11.1 mM glucose, supplemented with 3% FBS, 50 units/ml penicillin, 50 g/mL streptomycin).

2.6.2. Lymphocyte proliferation-viability assay

The PBMCs were supplemented with 100 U/mL penicillin (Gibco, USA), cultured in RPMI-1640 (Biochrom AG, Berlin, Germany) at 37 °C, and 10% fetal bovine serum (FBS) (Biochrom, Berlin, Germany). The concentration of cells was fitted to 500.000 cells/mL/well on a 24-well plate for two hours. Subsequently, the cells were treated with glucose in five concentrations that ranged from 25 to 1000 μg/mL of the hidroalcoholic extract of *Moringa oleifera* leaves made in culture medium, added to the PBMCs for 24. The cell viability was determined by MTT assay by using culture of cells in 96-well plates with 10% MTT reagent and 5 mg/mL of phosphate buffer solution. Then, the purple formazan salt, formed after Mitochondrial dehydrogenases metabolize MTT, was solubilized in 100 μL of dimethyl sulfoxide (DMSO), and absorbance was measured espectrophotometrically at 560 nm (Mosmann, 1983).

2.7. In vivo analysis

2.7.1. Experimental design

The diabetes type one was induced in 24 male Wistar rats aged 90 days (weighing 200 – 250 g) through intraperitoneal administration of 55 mg/kg (Mythili et al., 2004) body weight streptozotocin dissolved in sodium citrate 0.1 mol/L (pH 4.5). The animals with blood glucose $\geq$ 300 mg/dl after 7 days were used for the study. The animals were randomly distributed into 4 groups as follows (n = 6): group 1 (normal control - NC), group 2 (diabetic control - DC), group 3 (HE 500 mg/kg - HEC), group 4 (diabetic + 500 mg/kg HE - DHE). The Hidroalcoholic extract (HE) from *Moringa oleifera* leaves was suspended in solution of sodium chloride (0.9%) and the same solution was used as vehicle and administred to both control groups. Treatment was conducted by gavage for 45 days after confirmation of diabetes. During all experiments the animals were mantained in collective cages under dark/light cycles at 23 ± 1 °C and received food and water *ad libitum*. The research was conducted in accordance with internationally accepted principles for laboratory animal use and care.

2.7.2. Tissue preparation

The next day after the final treatment, the animals were anesthetized with halothane to euthanasia. The samples of liver and kidney tissues were placed on ice after removal homogenized (50 mM Tris HCl pH 7.4–1/10 w/v) and then centrifuged at 2000 g, at 4 °C, for 10 min. The supernatant (S1) was used for determination to Catalase (CAT), Glutathione S-Transferase (GST), non-protein thiol group (NPSH) and thiobarbituric acid reactive substances (TBARS) assay. Throughout the analysis procedures the samples were kept under ice.

2.7.3. Catalase (CAT) activity

The liver (1/10, w/v) and kidney (1/5, w/v) tissues homogenized in phosphate buffer (50 mM, pH 7.5) were centrifuged at 2000g to obtain the supernatant which was used for enzymatic activity. The methodology suggested by Nelson and Kiesow (D. P. Nelson & Kiesow, 1972) was used to determine CAT activity and was expressed in units/mg protein. Protein was determined according to Bradford (Bradford, 1976) method. The dye Coomassie Blue G-250 was used with bovine serum albumin as standard at absorbance of 595 nm.

2.7.4. Determination of glutathione-S-transferase activity (GST)

The 1-chloro-2–4-dinitrobenzene (CDNB) was used to GST activity. The activity was determined at 340 nm by the method Habig et al. (Habig et al., 1974). The S1 containing phosphate buffer (pH 7.4, 100 mM GSH; 100 mM CDNB) was used as substrate, and enzymatic activity expressed as nmol CDNB conjugated/min/mg protein.

2.7.5. Thiol non-protein (NPSH) determination

The Ellman (Ellman, 1959) reagent was used to estimated NPSH level spectrophotometrically. The samples from liver or kidney were homogenized and centrifuged at 10,000 rpm for 5 min at 4 °C. The supernatant from samples were used to NPSH measures and added 5,5́-dithiobis-2-nitrobenzoic acid (DTNB; 5 mM; for awaited 15 min at room temperature) and the reading was done by spectrophotometer at 412 nm. The results were expressed as μmol/g tissue.

2.7.6. Determination of lipid peroxidation

According to the method colorimetric described previously by Ohkawa et al. (Ohkawa et al., 1979) the measuring thiobarbituric acid reactive substances (TBARS) was determinated in liver and kidney. Briefly, 200 mL of samples of S1 from liver and kidney or MDA-malondialdehyde 0.03 mM, plus 200 mL of 8.1% sodium dodecylsulfate (SDS), and 750 mL of acetic acid solution (2.5 M HCl, pH 3.5) and 750 mL of 0.8% TBA were used to determine the TBARS in tissues, heated at 95 °C for 90 min. TBARS tissue levels were expressed as nmol MDA/mg protein.

2.7.7. Statistical analysis

The statistical analysis was performed using two-way ANOVA followed by Tukey post hoc test where appropriate. Differences were considered significant between groups at $p < 0.05$ using the GraphPad Prism 5 program.

3. Results and discussion

3.1. Bioguided isolation for antioxidant activity

In this work, we demonstrated the antyglicemic potential of *M. oleifera* leaves crude extract and bioguided the isolation of isoquercetrin (1), astragalin (2) and 3-O-caffeoylquinic acid (3) (Fig. 1) that showed high antioxidant potential.

The first step in the process was to isolate compounds from *M. oleifera* leaves with antioxidant capacity. Thus, at every step of purification were performed antioxidant assays based in Single Electron Transfer – SET (F-C and FRAP assays), Hidrogen Atom Transfer – HAT (ORAC assay) and both (DPPH and ABTS assays), ensuring a complete antioxidant characterization. With exception of ABTS assay, the fractions Fr-EtOAc and Fr-Ace showed the highest antioxidant capacities which was higher than that found for HE (Table 1) that indicates a concentration of bioactive molecules in fractions obtained from solid-liquid extraction by using solvents of medium polarity. Besides of potential in scavenge synthetic radicals, the results obtained from ORAC assay indicates that Fr-EtOAc and Fr-Ace can react with peroxyl radical that can reflect physiological alterations (Floegel et al., 2011).

The Fr-Hydro has also shown a high content of phenolic compounds and capacity of scavenge ABTS radical. The $ABTS^{+\cdot}$ assay is applicable to lipophilic and hydrophilic antioxidant systems, whereas DPPH assay is more indicate for hydrophilic systems because the radical is soluble in organic media. From obtained results it is possible to suggest a strong correlation between phenolic content and antioxidant activities, all of them showed a correlation coefficient higher than 0.84 and the higher correlation was observed between C-F and FRAP method ($r^2 = 0.984$) (Table 2). Floegel et al., 2011 suggest that phenolic compounds are a major contributor to antioxidan activity in foods. In previous study developed by our research group, Oldoni et al. (2019a, 2019b) reported that the main phenolic compounds identified by using HPLC in fractions obtained from HE technique was gallic acid, caffeic acid, rutin and quercetin. Fr-EtOAc and Fr-Ace showed contents 24 and 65 times higher than HE respectively for caffeic acid while Fr-EtOAc presented 70 times more quercetin than HE.

Fr-EtOAc and Fr-Ace showed the greatest antioxidant potential in most assays with no statistically significant difference among them. Additionally, Fr-EtOAC presented a chromatographic chemical profile

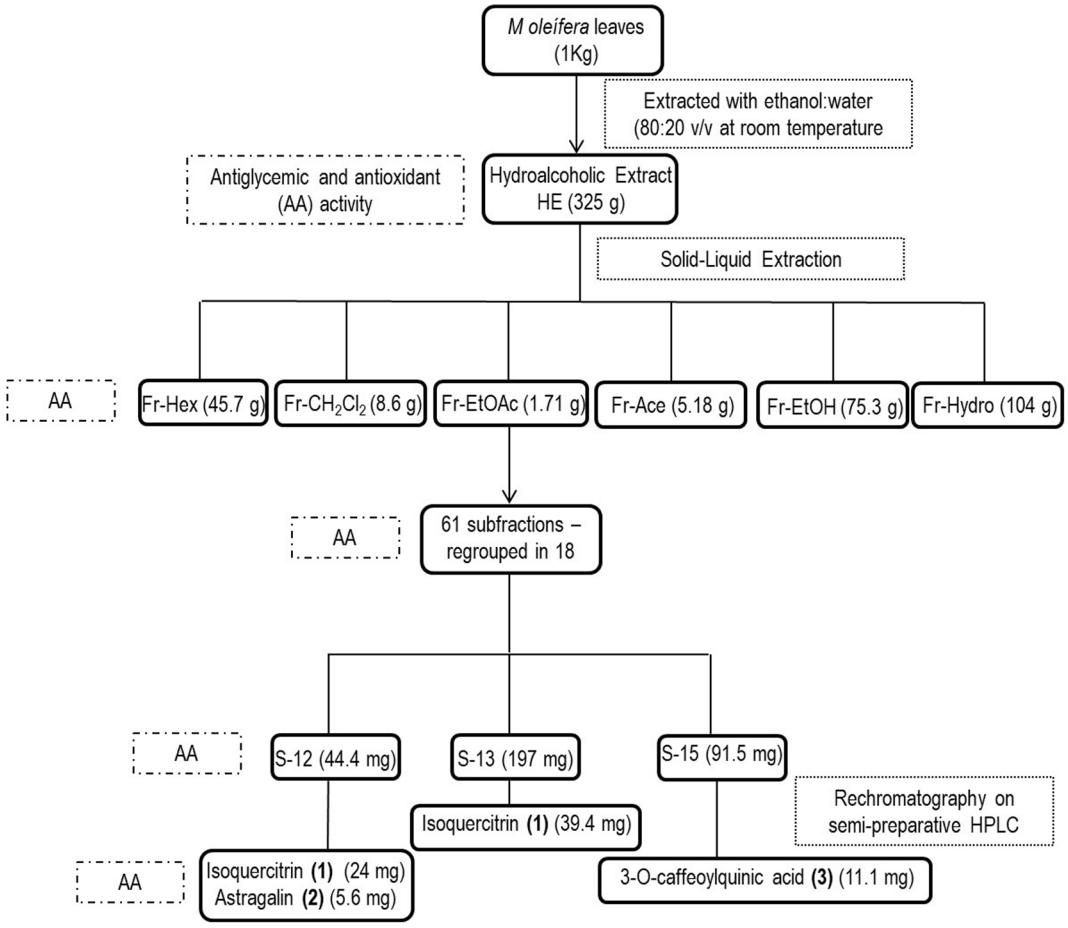

Fig. 1. Procedure for bioassay-guided isolation of compounds from *Moringa oleifera* leaves.

Table 1
Antioxidant activity of HE and fractions, obtained from *M. oleifera* leaves, evaluated by the F-C, FRAP, DPPH, ABTS and ORAC assays.

Sample	F-C assay	FRAP assay	DPPH assay	ABTS assay	ORAC assay
	(mg GAE/g)	(μmol Fe^{2+}/g)	(μmol TEAC/g)	(μmol TEAC/g)	(μmol TEAC/g)
HE	83.38 ± 1.2b	1293 ± 54.7 bc	341.6 ± 42.3b	928.5 ± 20.8 a	2932.0 ± 36.1c
Fr-Hex	n.d.	n.d.	95.61 ± 8.13c	190.5 ± 14.6 d	n.d.
Fr-CH_2Cl_2	41.70 ± 3.2c	537.0 ± 26.2 d	83.04 ± 1.52c	224.9 ± 3.94 d	n.d.
Fr-EtOAc	100.2 ± 5.7 a	1678 ± 68.8 a	526.7 ± 49.0 a	671.5 ± 72.9b	3560.6 ± 105 a
Fr-Ace	101.0 ± 1.6 a	1789 ± 89.8 a	435.7 ± 62.6 ab	705.2 ± 45.2b	3597.9 ± 98.9 a
Fr-EtOH	77.92 ± 2.6b	1162 ± 77.9c	373.5 ± 37.8b	475.6 ± 24.3c	3255.5 ± 91.9b
Fr-Hydro	99.61 ± 1.8 a	1436 ± 53.8b	526.4 ± 47.6 a	876.0 ± 43.4 a	2774.6 ± 104c

Values are expressed as mean ± SD (n = 3). Mean values with different letters, in the same column, for the same method of extraction, are significantly different ($p \leq 0.05$).
n.d. = not determined.
* Obtained from paired *t*-test ($p < 0.05$ indicates significant difference).

(data not shown) most favorable to the isolation of compounds. Thus, Fr-EtOAc was chromatographed over a sílica gel normal phase open column using a combination of mobile phase in increasing order of polarity that resulted in 61 subfractions which were regrouped in 18 subfractions, similar in color and Rf from TLC assay. All the subfractions were evaluated by antioxidant activities and the subfractions S-12, S-13 and S-15 showed the greatest potentials. The S-12 showed ability in scavenge synthetic and oxigen reactives species (DPPH, ABTS and ORAC) as well as was able to reduct ferric iron (Fe^{3+}) to ferrous iron (Fe^{2+}), which was higher than HE and Fr-EtOAc (Table 3). The antioxidant activity of S-12 was concentrated more than three times when compared wit HE in all assays evaluated.

The HPLC-$ABTS^{\bullet+}$ on-line assay was used as a tool to evaluate the antioxidant activity of individual compounds from subfractions using HPLC separation with post column reaction using stable radical ABTS and activity was measured and determined as a negative peak in chromatograms at 735 nm. Therefore it is possible to isolate biologically active compounds (Fig. 2A, 2B, 2D, 2E, 2G and 2H). The subfractions were rechromatographed in semi-preparative HPLC and S-12 was composed by isoquercitrin (1, 24 mg) and astragalin (2, 5.6 mg) (Fig. 2C) that showed DPPH as EC_{50} (Sridhar & Charles, 2019) values of 38.72 μg/mL and 144 μg/mL respectively. The S-13 afforded isoquercitrin (1, 39.4 mg) (Fig. 2F) and S-15 provided 3-O-caffeoylquinic acid (3, 11.1 mg) (Fig. 2I) that presented EC_{50} values of 30.3 μg/mL.

The highest potential of DPPH reduction was observed for isolated compound (1) 30.3 μg/mL (Table 3) which activity was six times higher

Table 2
The Correlation Coefficients between antioxidant activity and phenolic compounds.

	F-C (mg GAE/g)	FRAP (μmol Fe^{2+}/g)	DPPH (μmol TEAC/g)	ABTS (μmol TEAC/g)	ORAC (μmol TEAC/g)
F-C (mg GAE/g)	1				
FRAP (μmol Fe^{2+}/g)	0.98	1			
DPPH (μmol TEAC/g)	0.92	0.91	1		
ABTS (μmol TEAC/g)	0.84	0.81	0.82	1	
ORAC (μmol TEAC/g)	0.91	0.93	0.92	0.79	1

than that found for the HE (210.2 μg/mL) and closer than natural flavonoid quercetin (11.8 μg/mL). The main difference between flavonoids isolated from *M. oleifera* and quercetin is the glycosilation pattern that can lead to a reduction in the antioxidant activity (Valentová et al., 2014) but, on the other hand, although of remains unclear, the glycosides of quercetin seems to be more bioavailable than aglycone form (Hollman et al., 1996, 1997).

In fact, glycosilated flavonoids has excellent antioxidant properties and isolated compounds (1) and (2) (Fig. 3) present in their chemical structures at least two of three essentials characteristics that determine high antioxidant potential as: the o-dihydroxy (catechol) substituents in the B ring that are responsible by an antiradical activity; the presence of 4-oxo function and the double bond between carbons 2 and 3 that stabilize the radicals formed and delocalize π-electrons; and the presence of C-5 and C-7 hydroxyl groups (H in C-3 was substituted by an glycoside), that allow maximal radical scavenging capacity (Valentová et al., 2014).

3.2. Cell viability assay

The second step was to evaluate the cell viability and, sequentially, to evaluate the anthihyperglycemic potential of HE extract. In the MTT test it was possible to identify an increase in cell viability in the highest concentrations evaluated (250, 500 and 1000 μg/mL) with a significant difference when compared to the concentrations of 25 and 100 μg/mL of HE and control groups (0 and glucose) (Fig. 4). In summary, the results demonstrated that HE prolongs the life span of metabolically active cells in cell culture and HE treatment it was non toxic because did not kill or alter cell viability.

It is possible to suggest that isoquercetin, astragalin and chlorogenic acid contributed to the maintenance of cell integrity since they were isolated and identified as biological components in *M oleifera* extracts. Thus, the ingestion of the extracts can be beneficial to increase the probability of success against interventional agents that alter functions such as metabolism, growth, reproduction, including oxidative stress. In addition the positive result obtained from cell viability test provided the

Table 3
Antioxidant activity of HE, fractions and subfractions obtained from *M. oleifera* leaves.

Sample	FRAP	DPPH	ABTS	ORAC	EC_{50}
	μmol Fe^{2+}/g	μmol Trolox (TEAC)/g			(μg sample/mL)
HE	1293.5 ± 54.7 d	341.6 ± 42.3 d	928.5 ± 20.8 d	2932 ± 36.1 d	210.2 ± 3.16 a
Fr-EtOAc	1678.9 ± 68.8c	526.7 ± 49.0b	671.5 ± 72.9 e	3561 ± 105c	171.4 ± 1.20b
S-12	4998.0 ± 183 a	1066 ± 64.9 a	3430 ± 55.1 a	9328 ± 430 a	55.09 ± 2.01 e
S-13	1843.4 ± 60.1c	258.5 ± 14.0 d	2040 ± 162c	4554 ± 221b	135.4 ± 3.66 d
S-15	2379.7 ± 154b	511.4 ± 7.65c	2582 ± 88.4b	4460 ± 270b	151.2 ± 2.10c

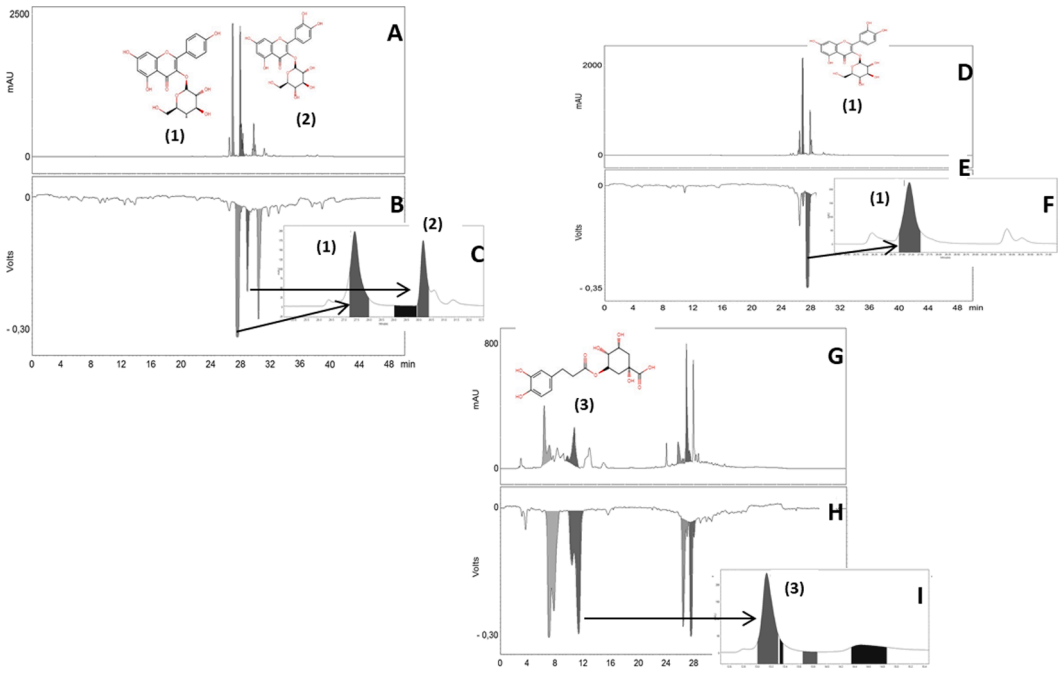

Fig. 2. HPLC- ABTS online chromatograms as positive and negative peaks and isolated compounds respectively: subfraction 12 - S-12 (A), (B) and (C); subfraction 13 - S-13 (D), (E) and (F); subfraction 15 - S-15 (G), (H) and (I).

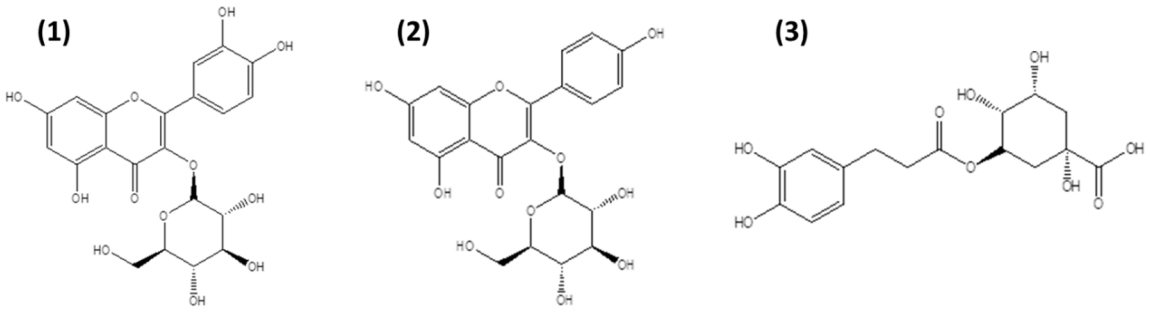

Fig. 3. Phenolic compounds isolated from *Moringa oleifera* leaves: Isoquercitrin **(1)**, Astragalin **(2)** and 3-O-caffeoylquinic acid **(3)**.

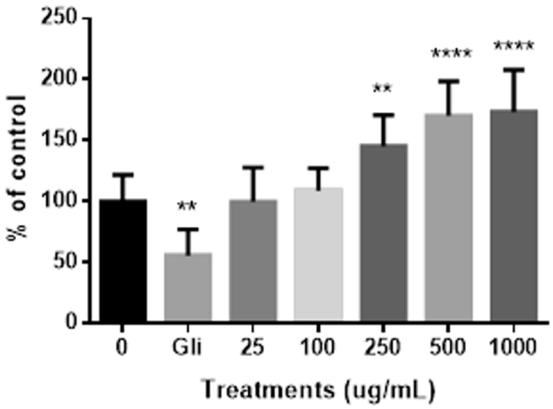

Fig. 4. Cell viability determined after 24 h by MTT assay in mononuclear cells of human peripheral blood (PBMCs) of controls 0 (zero HE, control), Gli (glucose, control) and treatment with *Moringa oleifera* hydroalcoholic extract (HE) 25 μg/mL to 1000 μg/mL HE. Data are presented as means ± SEM. **Indicates a significant difference from the control group (One-Way ANOVA, $p < 0.001$, $n = 5$). ****Indicates a significant difference from the control group (One-Way ANOVA, $p < 0.00001$, $n = 5$).

indication that the extract in a concentration of up to 1000 mg/mL can be used in the *in vivo* experiments.

3.3. Blood glucose and body weight

The blood glucose and body weight levels were assessed at the beginning and end of treatment (Fig. 5), and it was observed, at the end of 45 days of the study, significant differences among the groups. The blood glucose levels were significantly increased, and the body weight was significantly decreased in diabetic control groups (DC) ($P < 0.05$) when compared to the normal control group (NC) (Fig. 5A-B). However, the diabetic animals treated with hidroalcoholic extract of *M. oleifera* (DHE) decreased significantly the level of glucose when compared to the diabetic control group (DC). The *M. oleifera* per se (HEC) not exert effects on glucose levels or on body weight when compared to the NC. In addition, there were no significant differences in body weight when hidroalcoholic extract of *M.oleifera* was administered in the diabetics when compared with diabetic rats. Despite the variations, both control groups showed values within the normal range.

In weight gain resuts, the DHE group showed an increase of 2.9% in relation to the initial weight whiles the NC and HEC groups increased by 18.5 and 4.9%, respectively. The animals in the untreated diabetic group (DC) showed a reduction of approximately 11% in body weight. The insulin can act in weight gain since can causing muscle protein synthesis and lipogenesis (Ye, 2013). The decrease in insulin promotes a decrease in the activity of several metabolic pathways such as glycolysis, lipogenesis, glycogenesis, and other metabolic pathways are activated such as lipolysis, glycogenolysis and hepatic gluconeogenesis resulting in loss of body weight (D. L. Nelson & Cox, 2017).

The ROS radicals as superoxide and hydroxyl can be generated from hydrogen peroxide dismutation during hypoxanthine metabolism (Banerjee et al., 2019; Nukatsuka et al., 1990) when streptozotocin is used as diabetes inductor. This inductor is largely used because can to induce diabetes in animals and is known for its cytotoxicity in pancreatic islet β-cell. The consequences of the DNA mitochondrial fragmented thereby impairing the signalling function of beta cell mitochondrial metabolism and explain how streptozotocin is able to inhibit glucose-induced insulin secretion. Finally, some generation of ROS may accelerate the process of beta cell destruction when accompanied by streptozotocin (Banerjee et al., 2019). In addition, phenolic compounds present in *M oleifera* extract can act as insulin secretagogues to maintain glucose homeostasis upon hyperglycemia by targeting pancreatic β-cells.

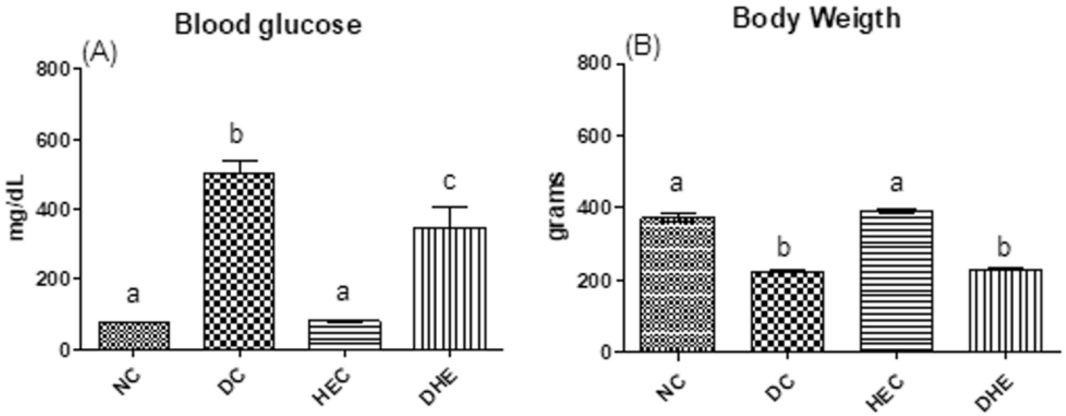

Fig. 5. Effect of *Moringa oleifera* on fasting blood glucose (A) and body weight (B) levels. Values are presented as mean (SD). Groups with different letters are statistically different ($p < 0.05$; $n = 6$). Groups: NC: normal control; DC: diabetic control; HEC: hidroalcoholic extract control; DHE: diabetic hidroalcoholic extract.

The fasting blood glucose (FBS) level was found to be significantly increased in diabetic rats induced by streptozotocin and diabetic rats treated with hidroalcoholic extract of *M. oleifera*. However, DHE group decreased significantly when compared to diabetic rats (DC) (Fig. 5A). This study is in agreement with Oyenihi et al (2019); Rahimi et al (2020) and Singh et al (2014) which demonstrate that the plants extract can be associated with a decrease in FBS level or use natural products in the management of diabetes. We can suggest possibles pathway to which the plant extract decrease the FBS. The first, is a possible interaction with key enzymes of glucose (such as hexokinase, phosphofructokinase and fructose 1,6-bisphosphatase) and glycogen metabolism (such as glycogen synthase and glycogen phosphorylase). Other, include the increase in the serum insulin level which mechanism involves removal of intracellular reactive oxygen species (ROS) in islet cells produced by streptozotocin by high concentration of glycosilated flavonoids and phenolic acid isolated from *M. oleifera* leaves (Isoquercitrin, Astragalin and 3-O-caffeoylquinic acid).

3.4. Antioxidants enzymes and markers of oxidative stress in liver and kidney tissues

The effect of hidroalcoholic extract from *M. oleifera* on the enzymes antioxidants catalase (CAT) and glutathione-S-transferase (GST) activities, and marker of oxidative stress as thiol non-protein (NPSH) level and measure of thiobarbituric acid reactive substances (TBARS) in liver and renal tissues are presented in Figs. 6, 7, 8 and 9, respectively.

The CAT activity in liver (Fig. 6A) and kidney (Fig. 6B) was significantly decreased in the diabetic rats (DC) when compared to the control rats (NC) ($P < 0.05$). The study with DHE increased CAT activity in the kidney in the dose of 500 mg/kg when compared to the DC group rats. Important to note that CAT activity subjected to the treatment with HEC *per se* not was altered in both types of tissues in the same dose of 500 mg/kg when compared to the NC groups.

From kidney tissue, it is possible to suggest that the animals in the diabetic group treated with *M. oleifera* showed sufficient activity to restore the enzymatic activity of CAT at the level of the control groups (NC and HEC) (Fig. 6B). In the liver tissue, the DHE group showed no significant difference from the diabetic group DC (Fig. 6A), and this can be attributed to the low dose of the plant extract used in the experiment. Pigeolet et al (1990) suggest that the decrease activity of catalase is inhibited by an excess of radical superoxide. In a study by Jaiswal et al. (2009) the aqueous extract of *M. oleifera* leaves of Indian origin on diabetic rats was evaluated and the treatment increased the activities of CAT, SOD and GST, together with a decrease in the level of TBARS, suggesting an antioxidant potential of *M. oleifera* extract.

In the diabetic control group, the results obtained for GST activity indicates a decrease in liver and an increase in kidney when compared to the NC group ($P < 0.05$). The HEC *per se* did not alter the GST activity in the dose 500 mg/kg in both tissues. The treatment with DHE was not able to prevent the decrease in GST activity in liver of diabetic rats but was efficient in the kidney. Additionally, the DHE group did not shown significant change in GST activity when compared to the HEC group in kidney.

Total GST activity showed metabolic activity in the cytosol of all tissues studied which was decreased in the diabetic rats group in liver (Fig. 7). What becomes clear, according to the decline in liver GST activty, is that diabetes toxicity involved a change in cellular redox status toward a state of oxidative (Pigeolet et al., 1990). Therefore, it can be assumed that the decrease in GST activity to be restricted, as evident by the intensification of lipid peroxidation (Jaiswal et al., 2009). The treatment with *M. oleifera* was not able to prevent this decrease in GST activity in liver of diabetic rats.

Whereas, our study indicate that the treatment with *M. oleifera* extract prevented the increase in GST activity in the kidney of diabetic rats, indicating a possible role of these antioxidant compounds in the plant (glycosilated flavonoids) in free radical inactivation and in the antioxidant defense. Antioxidant enzymes may increase their activities due to increased exposure to free radicals. This defense against free radicals is adaptable and considered by researchers to prove the occurrence of oxidative stress (Napierala et al., 2016).

The effects of *M. oleifera* extract in the liver tissue (Fig. 8A) demonstrated a significant decrease, at 99.5% confidence, in the quantification of nonprotein thiol group (NPSH) of diabetic animals treated with DHE when compared to diabetic rat group (DC). The extract *per ser* (HEC) did not change the NPHS level when compared to NC and DHE groups. In the kidney tissue, none of the groups showed significant changes in NPSH level (Fig. 8B). In the group of diabetic rats, an increase in NPSH levels in the liver was detected. This effect is likely to be a physiological compensatory effect. In fact, the levels of NPSH and reduced glutathione increase to counteract the high production of reactive oxygen species (Salgueiro et al., 2013). Glutationa is an intracellular antioxidant that acts as a buffer, which contributes for the thiol redox balance (Mbikay, 2012).

We also observed the increase in TBARS levels for the liver (Fig. 9A) and kidney (Fig. 9B) tissues of all diabetic animals when compared to NC, HEC and DHE groups. These changes are results of the reaction of free radicals with cell membrane structures and suggest that diabetes is present at 45 days of the experiment, denoted by the increase in oxidative stress in the diabetic group (DC) (Fig. 9A-B) (Carreño et al., 2017).

The malondialdehyde (MDA) is the index of lipid peroxidation in biological materials, considered a hallmark of oxidative stress. The ROS

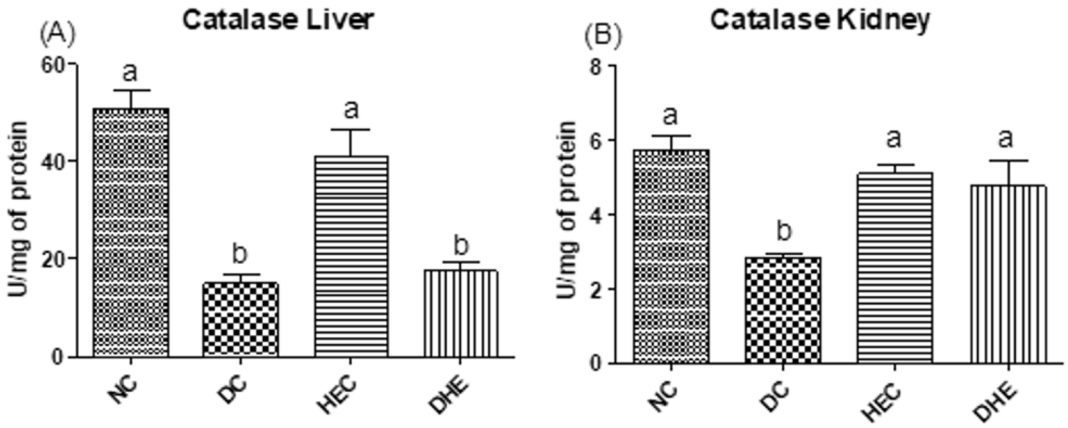

Fig. 6. Effect of *Moringa oleifera* on the CAT activity in the liver (A) and kidney (B) tissues. Values are presented as mean (SD). Groups with different letters are statistically different ($p < 0.05$; $n = 6$). Groups: normal control (NC); hidroalcoholic extract control (HEC); diabetic control (DC); diabetic hidroalcoholic extract (DHE).

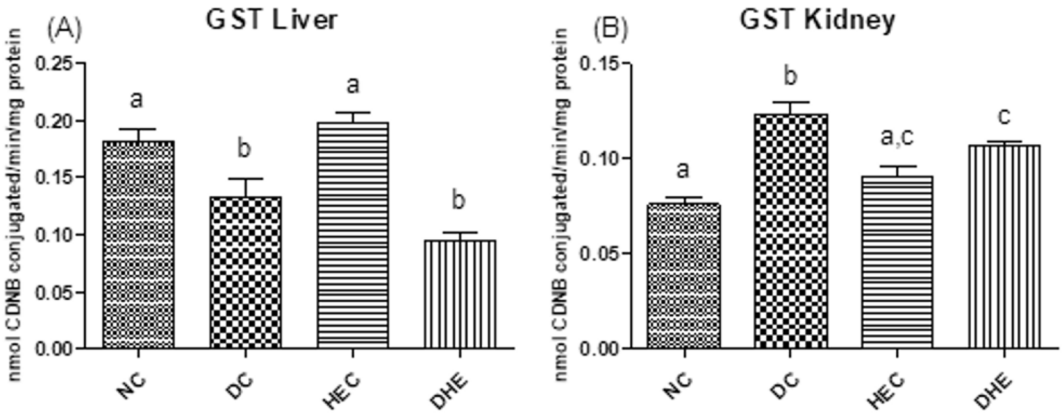

Fig. 7. Effect of *Moringa oleifera* on the GST activity in the liver (A) and kidney (B) tissues. Values are presented as mean (SD). Groups with different letters are statistically different ($p < 0.05$; $n = 6$). Groups: normal control (NC); hidroalcoholic extract control (HEC); diabetic control (DC); diabetic hidroalcoholic extract (DHE).

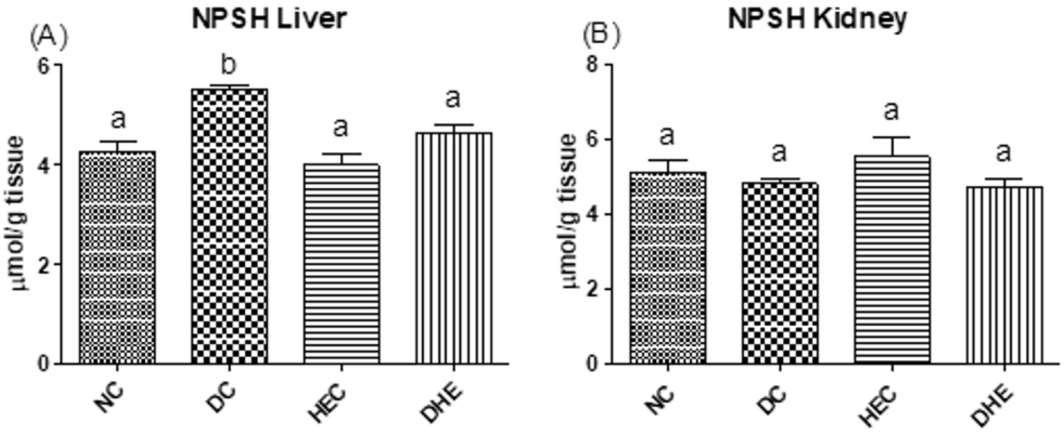

Fig. 8. Effect of *Moringa oleifera* on the NPSH levels in the liver (A) and kidney (B) tissues. Values are presented as mean (SD). Groups with different letters are statistically different ($p < 0.05$; $n = 6$). Groups: normal control (NC); hidroalcoholic extract control (HEC); diabetic control (DC); diabetic hidroalcoholic extract (DHE).

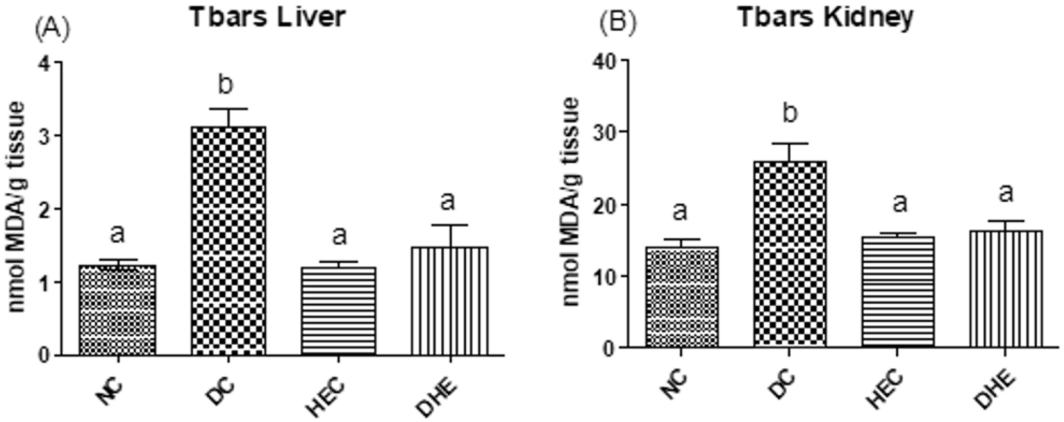

Fig. 9. Effect of *Moringa oleifera* on thiobarbituric acid reactive substances (TBARS) in liver (A) and kidney (B). Values are presented as mean (SD). Groups with different letters are statistically different ($p < 0.05$; $n = 6$). Groups: normal control (NC); diabetic control (DC); hidroalcoholic extract control (HEC); diabetic hidroalcoholic extract (DHE).

generation interact with polyunsaturated fatty acids, and leads to the formation of lipid products such as MDA and 4-HNE (4-hydroxynonenal). Damages of cell membrane might occurred due to oxidation of lipid membrane and cause the cell necrosis and inflammation (Gaschler & Stockwell, 2017). The HEC proved to be non-toxic and did not alter the levels of malondialdehyde when compared to the NC group. Thus, it is possible to suggest that the hidroalcoholic extract of *Moringa oleifera* leaves showed an antioxidant effect in liver and kidney, justified by the decrease in lipid peroxidation when submitted to the experimental model of diabetes.

From results obtained for antioxidants enzymes and markers of oxidative stress we observed that hidroalcoholic extract of *M. oleifera* leaves it was able to prevent, in diabetic rats, an oxidative damage in kidney and liver mainly because improved the antioxidant defense system and also decrease the peroxidation of lipids. The antioxidant potential can be intimately related to potent action of glycosilated flavonoids isoquercitrin (1) and astragalin (2) and phenolic acid chlorogenic (3) isolated and previously described in this study.

These molecules have therapeutic action and have been shown increasing attention due to health benefits and biological activities which increase the protective effects regarding different diseases. For Ahangarpour et al (2019) the structure of chlorogenic acid enables its activity similar to metformin in potentiating insulin action. An outcome of this hypothesis is that the administration of chlorogenic acid concomitant with meals, can decrease postprandial glycemia, thus promoting the production of GLP-1 reducing the risk of developing diabetes (Meng et al., 2013).

Kushwaha et al., 2014 when assessing the serum antioxidant effect of supplementation with *M. oleifera* leaves on markers of oxidative stress in postmenopausal women aged 45 to 60 years, stated that leaves decreased the amount of malondialdehyde by 16.3%. In this way, the bioactive compounds present in the plant increase the body's antioxidant level, decreasing the oxidative stress marker, that is, malondialdehyde.

Han et al (2019) evaluated the effect of astragalin in streptozotocin-induced diabetic mice with spermatogenic dysfunction. The authors stated in their study that astragalin has a direct protective effect on the injured testicle, inhibiting oxidative stress and regulating the inflammation of animals. Astragalin can contribute to glucose homeoastase through the influx of calcium into the K^{+}ATP, L-VDCC channels, mobilizing calcium from intracellular reserves and activating the PKC and PKA kinase proteins, leading to insulin secretion (Rey et al., 2019).

So, some points are relevant and should be evaluated in future investigations, in particular, a possible interaction with key enzymes of glucose (such as hexokinase, phosphofructokinase and fructose 1,6-bisphosphatase) and glycogen metabolism (such as glycogen synthase and glycogen phosphorylase), serum insulin concentration and, the concentration in the plasma and the effects of isoquercitrin (1) and astragalin (2) and phenolic acid chlorogenic (3) in antihyperglicemic model.

4. Conclusions

By the first time, the bioguided isolation of compounds with antioxidant activity from *M. oleifera* cultivated in Brazil resulted in the identification of two glycosylated flavonoids (isoquercitrin (**1**) and astragalin (**2**) and phenolic acid (3-*O*-caffeoylquinic acid (**3**). The antioxidant activity evaluated by reducing the initial concentration of DPPH in 50% indicated a promissor antioxidant potential of **1** and **3** that could be used as markers in *M. oleifera* extracts. Furthermore, the HE was able to enhance the antioxidant defense system and decrease the lipid peroxidation in liver and kidney. The identification of these bioactive compounds is important to improve the development and production of this natural product, and in the future, may be used as an antioxidant and as phytotherapic.

CRediT authorship contribution statement

Tatiane Luiza C. Oldoni: Supervision, Project administration, Conceptualization, Formal analysis, Writing - original draft. **Nathalie Merlin:** Methodology, Visualization, Investigation. **Thariane Carvalho Bicas:** Investigation. **Anaclara Prasniewski:** Investigation. **Solange Teresinha Carpes:** Funding acquisition. **Jociani Ascari:** Validation, Formal analysis, Writing - review & editing. **Severino Matias de Alencar:** Resources, Writing - review & editing. **Adna Prado Massarioli:** Investigation. **Margarete Dulce Bagatini:** Resources. **Rafael Morales:** Conceptualization. **Gustavo Thomé:** Conceptualization, Formal analysis, Writing - original draft.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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