Ethanol Extract of *Passiflora cincinnata* Seeds Posses Antidiabetic, Antiglycant, and Antioxidant Activities *in vitro*

Flávia Adaís Rocha dos Santos[1], Elaine Luiza Santos Soares de Mendonça[1], Felipe Cabral da Silva[1], Jadriane de Almeida Xavier[1*], J. P. Jose Merlin[2], Marília Oliveira Fonseca Goulart[1], H. P. Vasantha Rupasinghe[2*]

[1]Institute of Chemistry and Biotechnology, Federal University of Alagoas; Alagoas, Maceió, Brazil; [2]Department of Plant, Food, and Environmental Sciences, Faculty of Agriculture, Dalhousie University; Truro, New Scotland, Canada

This work aimed to investigate the antidiabetic, antiglycation, and antioxidant potentials of the ethanol extract of seeds of *Passiflora cincinnata* (EPCIN) *in vitro*. The EPCIN was evaluated from the following assays: total phenolic content (TPC – mg of Gallic Acid Equivalents (GAE)/g of dry extract), Radical Scavenging Assays (DPPH•, HOCl-scavenging assay), and protective effects against glycation of bovine serum albumin (BSA) with methylglyoxal (MGO) or a mixture of reducing sugars, fructose and glucose, as well as the potential for MGO capture by derivatization with ortho-phenylenediamine (OPD). To evaluate the antidiabetic activity of EPCIN *in vitro*, we used the assays of enzymes α-amylase (4 U/mL), α-glucosidase (0.25 U/mL), and dipeptidyl peptidase-4 (DPP-4 – 3.125 mU). The cell viability of EPCIN-pretreated normal human bronchial epithelial cells (BEAS-2B) alone or in the presence of the carcinogen 4-[(acetoxymethyl)nitrosamine]-1-(3-pyridyl)-1-butanone (NNKOAc) was measured using MTS assay. Quercetin (QCT), piceatannol (PIC), acarbose (ACB), and sitagliptin (STG) were used for comparison purposes. EPCIN had an average of TPC 157.0 ± 1.5 mg of GAE/g of dry extract, exhibited IC_{50} for DPPH• and HOCl of 11.9 ± 1.8 µg/mL and 6.9 ± 0.9 µg/mL, respectively. EPCIN and AMG inhibited the formation of advanced glycation end-products (AGE) with IC_{50} of 574 ± 8.7 and 31.9 ± 2.7 µg/mL for the initial stage and 542.6 ± 2.7 and 52.8 ± 8.1 µg/mL for the intermediate stage of glycation, respectively. EPCIN showed IC_{50} for α-amylase and α-glucosidase of 218.2 ± 15.9 µg/mL ($p<0,05$) and 242.0 ± 25 µg/mL ($p<0,05$), respectively. EPCIN did not show cytotoxicity for BEAS-2B cells at 10 and 50 µg/mL concentrations. In addition, it was also able to protect cultured human cells from oxidative stress caused by the NNKOAc at 100 µM. The *in vitro* evidence of the potential antioxidant, antiglycant, and antidiabetic effects warrants further investigation of the antidiabetic potential of *Passiflora cincinnata* seeds.

Keywords: Passion Fruit. Phytochemicals. Oxidative Stress. Type 2 Diabetes. Cytotoxicity.

Introduction

Since ancient times, phytochemicals have been used worldwide as alternative/complementary therapies to treat several pathologies due to the presence of biologically active compounds - secondary metabolites, sometimes biosynthesized, in plants, after some chemical, physical or biological injuries [1]. Among them, polyphenols have been highlighted, mainly regarding their antioxidant activity. These compounds, among other mechanisms of molecular action, have decreased NF-kB expression pathways, attenuating the excessive production of reactive oxygen species (ROS) and decreasing lipoperoxidation and damage of biomacromolecules. Additionally, they stimulate the NRF2 signaling pathway, which leads to an increase of antioxidant enzymes, such as superoxide dismutase (SOD), glutathione peroxidase (GPx), and catalase (CAT) [2].

Although polyphenols have well-established antioxidant activity, a particular group of non-flavonoid compounds, the stilbenes, has increased interest in the scientific community. Among the stilbenes - resveratrol, piceatannol (PIC), hesperidin, hesperetin, pterostilbene, polidatin, stilbestrol, and pinosylvine have shown multiple spectra of biological activities, with a special emphasis in PIC. PIC's relevance is probably

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Address for correspondence: Jadriane Almeida Xavier. Institute of Chemistry and Biotechnology, Federal University of Alagoas, Maceio, Zip code: 57072-970, Brazil. E-mail: jadrianexavier@iqb.ufal.br. H.P. Vasantha Rupasinghe. Department of Plant, Food, and Environmental Sciences, Faculty of Agriculture, Dalhousie University, 50 Pictou Road, Truro, NS B2N 5E3, Canada, e-mail: vrupasinghe@dal.ca.
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related to the presence of the catechol moiety [3-5]. In addition, researchers have identified the presence of PIC in the genus *Passiflorae*, which comprises more than 500 species, found predominantly in tropical and subtropical regions of the world [6-8].

In northeastern Brazil, the species P*assiflora cincinnata*, popularly known as "wild passion fruit" or "passion fruit of the Caatinga", has been less explored compared to other species because it has a specific sensory analysis, with unusual organoleptic characteristics, mainly regarding the pulp of its fruits, being considered to have a stronger flavor than the other species. However, it is worth mentioning the natural beauty of its flowers and its resistance to pests and hydric deficit [8]. In addition, the literature is scarce regarding their chemical composition and biological profile.

In this context, the objective of the present work was to evaluate the *in vitro* antioxidant, antiglycant, and antidiabetic activities of the ethanol extract of the seeds of *Passiflora cincinnata* (EPCIN). To the best of our knowledge, no study has been conducted so far reported concerning the seeds of Brazilian *P. cincinnata* as an antidiabetic agent through the association of α-glucosidase and DPP-4 inhibition mechanisms, not even as an antiglycant in the initial and intermediate stages of glycation. In addition, the cytotoxicity of EPCIN was studied in BEAS-2B cell lines, with and without the oxidative stress-causative carcinogen NNKOAc.

Materials and Methods

Preparation of the Ethanol Extracts of *Passiflora cincinnata* Seeds

We collected the unripe fruits of *Passiflora cincinnata* in November 2019 in a native forest in Olho d'Água do Casado, Alagoas. The seeds were separated from the fruit pulp, washed with distilled water, and dried in an oven at 50 °C for 48 h. The ethanol extract of *Passiflora cincinnata* seeds (EPCIN) was obtained according to the method described by Xavier and colleagues [6].

Total Phenolic Content (TPC)

The TPC was estimated using the Folin–Ciocalteu (FC) method, as described by Cicco [9], with some modifications. Briefly, 180 µL of deionized water, 300 µL of FC reagent, and 2.4 mL of 5% sodium carbonate (w/v) were added to 120 µL of diluted samples. After incubation in a water bath at 40 °C in the dark for 20 min, the absorbance of the resulting mixture was measured at 760 nm using a UV–Vis spectrophotometer (Agilent 8453). The results were expressed as milligrams of gallic acid equivalents (mg GAE) per gram of dry extract.

Radical Scavenging Assay DPPH•

The antioxidant activity of EPCIN was determined using the DPPH• method [10]. Briefly, aliquots of 0.30 mL of sample dissolved in ethanol (5–25 µg/mL) were mixed with 2.70 mL of DPPH• solution (40 µg/mL in methanol). After incubation in the dark for 30 min, the absorbance was read at 516 nm, using a UV–Vis spectrophotometer (Agilent Technologies, Santa Clara, CA, USA). Results were expressed as the half-maximal inhibitory concentration (IC_{50}) in µg/mL.

Hypochlorous Acid (HOCl) Elimination Assay

The HOCl elimination activity of EPCIN and was determined using the previously described method [11]. Briefly, a new HOCl solution (30 µM) was prepared in 100 mM phosphate buffer (pH 7.4). Then, in a 96-well plate, the following reagents were added at the indicated final concentrations: buffer solution (100 mM, pH 7.4), EPCIN (1, 5, 10, 25, 50, 100, 200, and 300 µg/mL), dihydrorodamine (DHR) (5 µM) and HOCl (5 µM). QCT and PIC were used for comparison purposes. The fluorescence measurements were performed in a microplate reader (Infinite® 200 PRO, TECAN, Männedorf, Switzerland), at 37 °C, at wavelengths of 505 ± 10 nm and 530 ± 10 nm, for excitation and emission, respectively. The results were expressed as IC_{50} in µg/mL.

Measurement of Intracellular ROS Level

The generation of intracellular ROS in BEAS-2B after treatments with EPCIN was measured as described previously [12]. The cells, pre-treated with EPCIN for 3 h, were exposed to the carcinogen NNKOAc, for 3 h or alone in different experimental groups. DMSO (0.5%) was utilized as a control. ROS was quantified after treatments using the 2',7'-dichlorofluorescein diacetate dye (DCFH-DA) at a final concentration of 5 μM followed by 40 min incubation at dark. Fluorescence measurements were performed in a microplate reader (Infinite® 200 PRO, TECAN, Männedorf, Switzerland), at 37 °C, at wavelengths of 485 nm and 535 nm, for excitation and emission, respectively.

Cell Cultures and Cell Viability Assay

BEAS-2B cells were cultured with LHC-9 media at 37 °C in an incubator with CO_2 (5%). Culture flasks (polystyrene T75) were pre-coated with a mixture of fibronectin (0.01 mg/mL), bovine collagen type I (0.03 mg/mL), and bovine serum albumin (0.01 mg/mL) dissolved in the LHC-9 medium overnight. The MTS assay was used to perform the cell viability assay [13]. For 24 hours, different concentrations of EPCIN were used to examine the viability of the cells. Next, an MTS reagent was applied, and the cells were incubated for 3 hours in the dark. A microplate reader (Infinite® 200 PRO, TECAN, Männedorf, Switzerland) was used, and the absorbance was recorded at 490 nm.

α-Amylase Inhibition Assay

The α-amylase inhibition assay was performed using a previously described method [14] with minor modifications. EPCIN and PIC, in different concentrations, were prepared in 0.01 M potassium phosphate buffer (pH 6.8) containing 8% ethanol. All other solutions were prepared in buffer only. ACB was used as a positive control. The reaction system consisted of adding the samples, α-amylase from porcine pancreas (4 U/mL), and after 10 min of incubation at 37 °C, the substrate 2-chloro-4-nitrophenyl-α-D-maltotrioside (5 mM) was added and then incubated for more 30 min. The reaction was terminated by adding a trisodium phosphate solution of pH 11 (1%, w/v). The amount of 2-chloro-4-nitrophenol released was measured spectrophotometrically at 405 nm in a microplate reader (Infinite® 200 PRO, TECAN, Männedorf, Switzerland).

α-Glucosidase Inhibition Assay

The α-glucosidase inhibition assay was performed using a previously described method [15] with minor modifications. EPCIN and PIC, in different concentrations, were prepared in 0.01 M potassium phosphate buffer (pH 6.8) containing 2.5% ethanol. All other solutions were formulated in buffer only. The reaction system consisted of adding a sample, alpha-glucosidase enzyme (0.25 U/mL), and 4-nitrophenyl-α-D-glucopyranoside (5 mM) substrate. The mixture was then incubated at 37 ◦C for 15 min, and the reaction was stopped by adding sodium carbonate solution (0.2 M). Acarbose (ACB) was used for comparison purposes. The amount of p-nitrophenol (PNP) released was measured spectrophotometrically at 405 nm in a microplate reader (Infinite® 200 PRO, TECAN, Männedorf, Switzerland).

Dipeptidyl Dipeptidase Enzyme (DPP-4) Inhibition Assay

The DPP-4 inhibition assay was performed according to an established method [14]. STG, a standard DPP-4 inhibitor, was used to compare the effectiveness of PESE and PIC. Briefly, In a 96-well plate, the following reagents were added at the indicated final concentrations: the sample at different concentrations, DPP-4 human recombinant enzyme solution (3.125 mU), and Gly-Pro-7-amido-4-methylcoumarin hydrobromide substrate (2.5 μM). The reaction mixture was incubated for

30 min in the dark at 37 °C. Then, the fluorescent product was recorded using the microplate reader (Infinite® 200 PRO, TECAN, Männedorf, Switzerland) at wavelengths of 350 nm and 450 nm for excitation and emission, respectively.

Inhibition of Advanced Glycation End Products (AGE) Formation

The formation of AGEs was evaluated in the initial (BSA, glucose, and fructose) and final stages of glycation (system containing BSA and MGO). This assay was based on previous methods [6]. Aminoguanidine (AMG) was used as a positive control. We used a mixture of fructose and glucose-reducing sugars to evaluate the initial stage. The reaction system consisted of the addition of adequate volumes of EPCIN or AMG solutions (at different concentrations), D-fructose (200 mM), D-glucose (200 mM), and bovine serum albumin (BSA, 3 mg/mL) in a total volume of 1.5 mL. EPCIN was dissolved in ethanol. All other solutions were dissolved in 0.05 M potassium phosphate buffer (pH 7.4) containing NaCl (100 mM) and NaN3 (0.02% w/v). The reaction system was incubated in the dark at 37 °C for 7 days with constant stirring. For the evaluation of the intermediate stage, the same procedure was followed; however, MGO was used instead of the mixture of reducing sugars. The fluorescent AGEs were measured using a microplate reader (Infinite® 200 PRO, TECAN, Männedorf, Switzerland) at ʎex= 360 and ʎem = 440 nm. AMG, a known AGE formation inhibitor, was used as a standard.

Methylglyoxal Capture Potential

MGO capture was evaluated by derivatization with o-phenylenediamine (OPD) using a previously described procedure [6]. The reaction system was composed of MGO, phosphate buffer (negative control), or EPCIN (2 mg/mL) or AMG (2 mM, positive control) incubated at 37 °C for 1 h. After incubation, the OPD solution (4 mM) was added, and the tubes were kept for 30 min for the derivatization reaction between MGO and OPD to complete. Then, the formation of the derived product 2-methylquinoxyline (2-MQ) was monitored by high-performance liquid chromatography (HPLC) and detected at 315 nm. Formic acid (0.1% v/v, solvent A) and methanol (solvent B) were used as a mobile phase, with a flow rate of 1.0 mL/min. The linear gradient for elution was starting at 5% of solvent B, 0–3 min, 5 to 50% B; 3–16 min, isocratic in 50% B; 16–17 min, 50–90% B; 17–19 min, isocratic in 90% B and 19–19.5 min, 90–5% B. The percentage of MGO capture was calculated concerning the peak area corresponding to the 2-MQ product in the systems with and without treatment.

Statistical Analysis

All analyses were performed in triplicate (n = 3) using Graph-Pad Prism software (GraphPad Software Inc., San Diego, CA, USA). Data were presented as the mean ± standard deviation (SD), and analyses of variance, one-way ANOVA, followed by Tukey test, and $p \leq 0.05$ were considered significant between experimental groups.

Results and Discussion

The EPCIN yield was 1.9% from 12 g of dried and ground seeds. Extraction of extract yields is crucial in assessing the cost and benefits associated with potential nutraceutical development. Xavier and colleagues [7], when studying the ethanol extraction of the seeds of *Passiflora edulis*, obtained 4 times higher yields (7.9%). The degree of maturation of the fruit seems to be a factor that could influence the composition of the seeds.

Table 1 shows the levels of total phenols and antioxidant activity of EPCIN compared to QCT. Particularly concerning the DPPH• and HOCl elimination assays, we observed that although the antioxidant capacity of EPCIN was lower than that presented by the QCT (control), the IC_{50} values are close to those proposed by the standard.

Table 1. Total phenol content (TPC) and antioxidant potential of the ethanolic extract of seeds of P*assiflora cincinnata* in comparison to quercetin (QCT).

Sample	TPC dry extract of EAG (mg/g)	DPPH• IC_{50} (µg/mL)	HOCl IC_{50} (µg/mL)
EPC	157.0 ± 1.5	11.9 ± 1.8	6.9 ± 0.9
QCT	-	5.5 ± 0.6 (18.1)	2.2 ± 0.3 (7.2)

EPCIN: ethanolic extract of seeds of *Passiflora cincinnata*; QCT: Quercetin; DPPH•: 1,1-Diphenyl-2-Picrylhydrazyl; GAE: Gallic Acid Equivalents. Results in Parentheses (µM).

Table 2. *In vitro* antidiabetic activity of ethanol extracts from seeds of *Passiflora cincinnata* and Piceatannol (PIC), in comparison with acarbose (ACB) and sitagliptin.

Sample	α- Amylase - IC_{50} (µg/mL)	α- Glucosidase - IC_{50} (µg/mL)	DPP-4 - IC_{50} (µg/mL)
EPCIN	218.2 ± 15.9[c]	242.0 ± 25.1[b]	NA
PIC	85.9 ± 1.8 (339.4)[b]	21.5 ± 8.3 (88.0)	1,300.3 ± 20.0(5,323.9)
ACB	0.6 ± 0.1 (0.9)[a]	251.6 ± 4.5 (389.7)	-
STG	-	-	0.005 ± 0.0005 (0.01)

EPCIN: ethanol extract of seeds of *Passiflora cincinnata;* PIC: Piceatannol; ACB: acarbose; STG: sitagliptin; NA, it was not possible to calculate the IC_{50}; Data in parentheses (µM).

Santos and colleagues [6], when evaluating the antioxidant activity of the ethanol extract of *Passiflora edulis* seeds, observed a TPC of 227 ± 3.9 mg of GAE/g, approximately 2x that observed in the present study, which was reflected in the antioxidant activity in scavenging DPPH• (20.4 ± 2.1 µg/mL), with lower activity than EPCIN; however, the antioxidant activity, obtained through the ability to eliminate HOCl, of *Passiflora edulis* (1.7 ± 0.3 µg/mL) was approximately 4 times superior to that of EPCIN. The results suggest that EPCIN may be an essential source of phenolic compounds and that EPCIN has antioxidant activity, decreasing the formation of ROS and other metabolites.

The antidiabetic potential of EPCIN can be analyzed through the ability to inhibit α-amylase and α-glucosidase, in addition to the DPP-4 enzyme. This study used acarbose (ACB) and sitagliptin (STG) for comparison purposes. EPCIN showed a concentration-dependent activity against α-amylase and α-glucosidase inhibition, and the IC_{50} values are shown in Table 2. EPCIN and PIC, one of the compounds present in the seeds of *Passiflora cincinnata*, showed higher potential towards α-glucosidase than α-amylase. We noted that EPCIN showed toward α-glucosidase an IC_{50} value equivalent to that presented by ACB. However, it caused a low α-amylase inhibition. On the other hand, PIC showed excellent α-glucosidase inhibition potential, with IC_{50} about 4.5 times lower than ACB, suggesting that PIC plays an essential role in the antidiabetic potential of EPCIN.

BSA glycation was performed in the presence and absence of EPCIN. In the initial stage, IC_{50} values presented by EPCIN and AMG were 574.4 ± 8.7 and 31.9 ± 2.7 µg/mL, respectively. For the intermediate stage, where BSA was treated with MGO, the IC_{50} values were 542.6 ± 2.7 and 52.8 ± 8.1 µg/mL, respectively. EPCIN inhibited the formation of fluorescent AGEs; however, AMG showed higher activity when compared to EPCIN. Gomes and colleagues [16] evaluated

the hydroethanolic extract of *P. cincinnata* aerial parts. They observed a 68.17% inhibition of AGEs formation for a concentration of 10 mg/mL, while Santos and colleagues [6] found an IC_{50} of 360 µg/mL for the ethanol extract of *P. edulis* seeds.

When investigating the MGO capture potential in the reaction system, after 1 h incubation, EPCIN could capture 47.9% of the MGO present in the solution, while AMG presented 89.2% of MGO capture. The results show that EPCIN may act in the intermediate step of the glycation reaction, stabilizing the dicarbonyl species and thus inhibiting the response with amino groups of biomolecules through the formation of AGEs [17].

It has been recognized that when the cell viability is greater than 90%, the sample is considered non-toxic at a given dose. Figure 1A shows a dose-responsive decline in cultured BEAS-2B cells with increasing concentrations of EPCIN ($p \leq 0.05$). EPCIN did not alter cell viability up to a concentration of 50 µg/mL. Xavier and colleagues [7], when testing the cell viability (human placental HTR-8/SVneo) in the presence of the ethanol extract of *Passiflora edulis* seed, observed that there was no reduction in cell viability, even at the concentration used of 100 µg/mL.

Additionally, Figure 1B shows the effects of EPCIN on the BEAS-2B cells exposed to NNKOAc (100 µM), evidencing that the carcinogen was able to stimulate the production of ROS and that the pre-treatment with EPCIN was able to protect cultured BEAS-2B cells against oxidative stress. EPCIN was also added to cells in the absence of NNKOAc, and again no increase in ROS production was observed when compared to the control.

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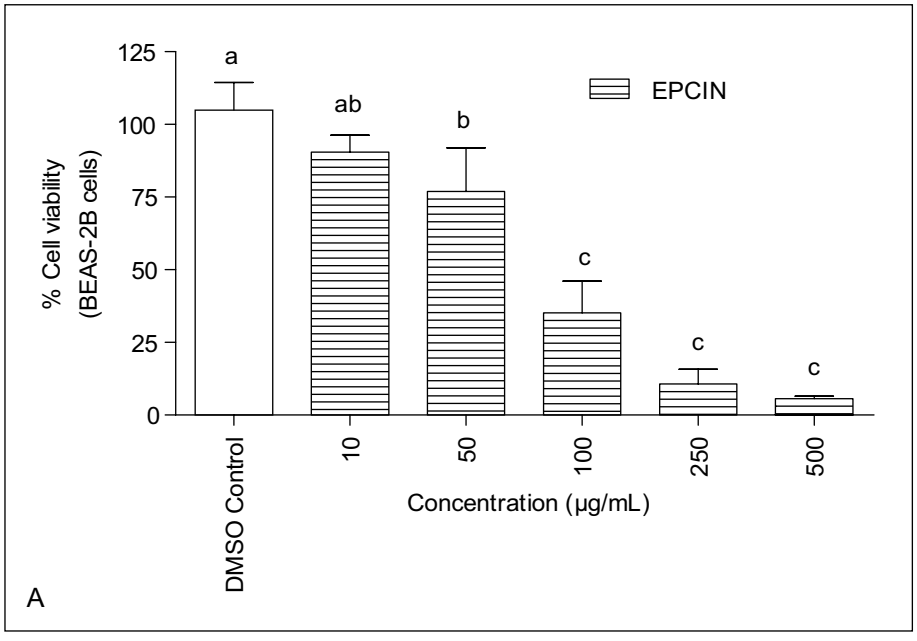
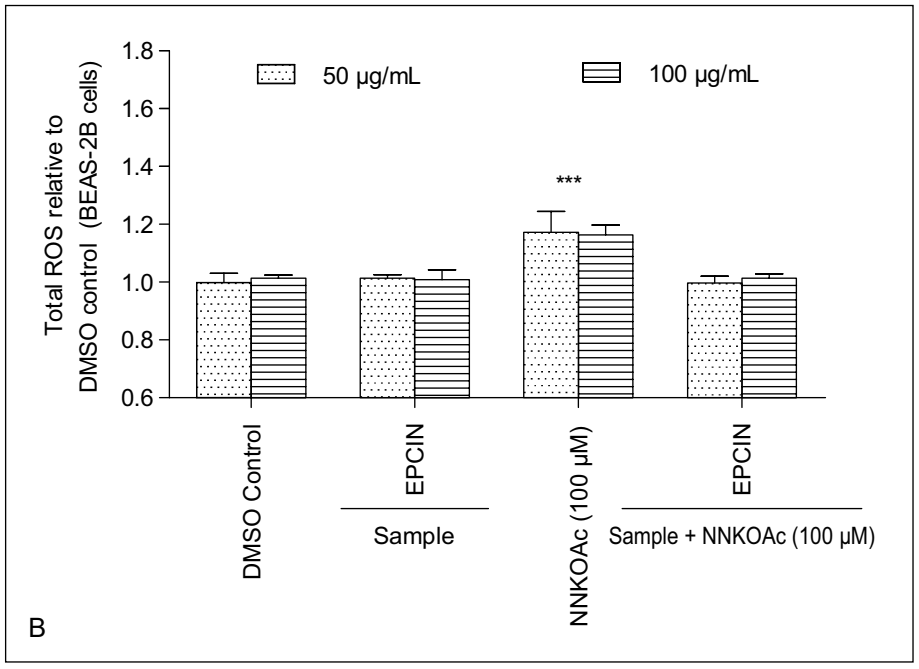
Figure 1. Dose-dependent effects of the ethanol extract of seeds of *Passiflora cincinnata* (EPCIN) on the viability of normal human bronchial epithelial cells (BEAS-2B) (A). The relative amount of ROS evaluated in normal human bronchial epithelial cells (BEAS-2B), after exposure to carcinogen alone or with pre-treatment of ethanol extract of seeds of Passiflora cincinnata (B) at concentrations of 50 and 100 µg/mL.





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Conclusion

EPCIN showed potential antioxidant, antiglycant, and antidiabetic effects. Furthermore, EPCIN was shown to be non-toxic to BEAS-2B cells at the concentration effective to exhibit antidiabetic and antioxidant activities, in addition to protecting the BEAS-2B cells from oxidative stress caused by NNKOAc. Based on this study, further studies need to be conducted using experimental animal models to assess the extract's safety and confirm its antioxidant, antiglycant, and antidiabetic activities. In conclusion, EPCIN has the potential to be used as an alternative therapy for managing type 2 diabetes mellitus.

References

1. Leal AEBP, de Oliveira AP, Santos RFD, Soares JMD, Lavor EM et al. Determination of phenolic compounds, *in vitro* antioxidant activity, and characterization of secondary metabolites in different parts of *Passiflora cincinnata* by HPLC-DAD-MS/MS analysis. Nat Prod Res. 2020 Apr;34(7):995-1001.
2. Ajit D, Simonyi A, Li R, Chen Z, Hannink M et al. Phytochemicals and botanical extracts regulate NF-κB and Nrf2/ARE reporter activities in DI TNC1 astrocytes. Neurochem Int. 2016 Jul;97:49-56.
3. Krambeck K et al. Identification and quantification of stilbenes (piceatannol and resveratrol) in *Passiflora edulis* by-products. Pharmaceuticals (Basel) 2020;20(13):73.
4. Seyed MA et al. Comprehensive review on the chemotherapeutic potential of piceatannol for cancer treatment, with mechanistic insights. J Agric Food Chem 2016;64(4):725-737.
5. Son PS et al. Piceatannol, a catechol-type polyphenol, inhibits phorbol ester-induced NF-kB activation and cyclooxygenase-2 expression in human breast epithelial cells: Cysteine 179 of IKKβ as a potential target. Carcinogenesis 2010;31(8):1442-1449.
6. dos Santos FAR, Xavier JA, da Silva FC, Merlin JPJ, Goulart MOF, Rupasinghe HPV. Antidiabetic, antiglycation, and antioxidant activities of ethanolic seed extract of *Passiflora edulis* and piceatannol *in vitro*. Molecules 2022;27(13):4064.
7. Xavier JA et al. Anti-zika virus effects, placenta protection and chemical composition of *Passiflora edulis* seeds ethanolic extract. Journal of the Brazilian

Chemical Society [online]. 2022;33(7):701-714. [Accessed 29 July 2022].
8. Brandão LEM, Nôga DAMF, Dierschnabel AL, Campêlo CLDC, Meurer YDSR et al. *Passiflora cincinnata* extract delays the development of Motor Signs and Prevents Dopaminergic Loss in a Mice Model of Parkinson's Disease. Evid Based Complement Alternat Med. 2017;2017:8429290.
9. Cicco N, Lanorte MT, Paraggio M, Viggiano M, Lattanzio V. A reproducible, rapid and inexpensive folin-ciocalteu micro-method in determining phenolics of plant methanol extracts. Microchem J. 2009;91:107-110.
10. Xavier JA, Valentim IB, Camatari FOS, de Almeida AMM, Goulart HF et al. Polyphenol profile by uhplc-Ms/Ms, anti-glycation, antioxidant and cytotoxic activities of several samples of propolis from the Northeastern semi-arid region of Brazil. Pharm Biol. 2017;55:1884-1893.
11. Lucas M, Freitas M, Xavier JA, Moura FA, Goulart MOF et al. The scavenging effect of curcumin, piperine, and their combination against physiological relevant reactive pro-oxidant species using *in vitro* non-cellular and cellular models. Chem Pap. 2021;75:5269-5277.
12. Kido LA, Hahm E-R, Kim S-H, Baseggio AM, Cagnon VHA et al. Prevention of prostate cancer in transgenic adenocarcinoma of the mouse prostate mice by yellow passion fruit extract and antiproliferative effects of its bioactive compound piceatannol. J Cancer Prev. 2020;25:87-99.
13. Sekhon-Loodu S, Rupasinghe HPV. Evaluation of antioxidant, antidiabetic and antiobesity potential of selected traditional medicinal plants. Front Nutr. 2019;6:53.
14. Rabbani N, Xue M, Thornalley PJ. Dicarbonyl stress, protein glycation, and the unfolded protein response. Glycoconjugate Journal 2021;38(3):331-340.
15. Rudnicki M et al. Antioxidant and antiglycation properties of *Passiflora alata* and *Passiflora edulis* extracts. Food Chemistry 2007;100(2):719-724.
12. Merlin JPJ, Dellaire G, Murphy K, Rupasinghe HPV. Vitamin-containing antioxidant formulation reduces carcinogen-induced DNA damage through ATR/Chk1 signaling in bronchial epithelial cells *in vitro*. Biomedicines 2021;9(11):1665.
13. Merlin JPJ, Dellaire G, Mathavarajah S, Murphy K, Rupasinghe HPV. A dietary antioxidant formulation ameliorates DNA damage caused by γ-irradiation in normal human bronchial epithelial cells *in vitro*. Antioxidants 2022;11:1407.
14. de Silva ABKH, Rupasinghe HPV. Polyphenols composition and anti-diabetic properties *in vitro* of Haskap (*Lonicera caerulea L.*) berries in relation to cultivar and harvesting date. J Food Compos Anal. 2020;88:103402.
15. Sekhon-Loodu S, Rupasinghe HPV. Evaluation of antioxidant, antidiabetic and antiobesity potential of selected traditional medicinal plants. Front Nutr. 2019;6:53.
16. Gomes AC et al. Antioxidant and antiglycation activities and inhibitory action of *Passiflora cincinnata* on collagenase, elastase and tyrosinase: *in vitro* and *in silico* study. Biocatalysis and Agricultural Biotechnology 2022;44:102464. http://dx.doi.org/10.1016/j.bcab.2022.102464.
17. Rabbani N, Xue M, Thornalley PJ. Dicarbonyl stress, protein glycation and the unfolded protein response. Glycoconjugate Journal 2021;38(3):331–340.