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Purple tea: chemical characterization and evaluation as inhibitor of pancreatic lipase and fat digestion in mice†

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A variety of the classic green tea plant, *Camellia sinensis*, was developed and is exclusive to Kenya. Due to high content of anthocyanin polyphenols in its leaves, the beverage obtained from this variety is purple in color and is the origin of the name purple tea. This work had two main purposes. The first one was to identify and quantify the major anthocyanin polyphenols in a hot water aqueous extract of the purple tea leaves. The second one was to test the hypothesis if this extract is capable of inhibiting triglyceride absorption considering that anthocyanin polyphenolics have been frequently associated to antilipidemic effects. Parallel experiments were always done with a similar green tea extract for comparison purposes. The antioxidant, anti-inflammatory, and cytotoxic activities of both tea varieties are similar. The purple tea extract, however, was strongly inhibitory toward the pancreatic lipase (minimal IC_{50} = 67.4 μg mL^{-1}), whereas the green tea preparation was a weak inhibitor. Triglyceride digestion in mice was inhibited by the purple tea extract starting at 100 mg kg^{-1} dose and with a well-defined dose dependence. Green tea had no effect on triglyceride digestion at doses up to 500 mg kg^{-1}. The latter effect is probably caused by several components in the purple tea extract including non-anthocyanin and anthocyanin polyphenols, the first ones acting solely *via* the inhibition of the pancreatic lipase and the latter by inhibiting both the lipase and the transport of free fatty acids from the intestinal lumen into the circulating blood. The results suggest that the regular consumption of Kenyan purple tea can be useful in the control of obesity.

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Introduction

The beverage prepared with green tea (*Camellia sinensis*), whose origins go back to ancient China, has become increasingly popular worldwide. Green tea has been extensively studied and its regular consumption is correlated with many benefits for human health.[1] More recently, a new variety of green tea has been described, which grows under cooler conditions, at elevations between 4500 and 7500 feet. This exposes the plants to more intense sun rays, causing them to produce higher levels of protective anthocyanins, which confer a purple color to the leaves.[2,3] Due to the high content of anthocyanins in the leaves, the beverage obtained from this new variety has the purple color, which is the origin of the name purple tea. It must be emphasized that purple tea is not a tea type characterized by its manufacturing method. It is a different cultivar, which is manufactured according to the same methods used in the preparation of green tea.

The bioactivities of green tea, such as antioxidant, antibacterial, antitumoral, anti-inflammatory, antidiabetic, antiproliferative, and anti-atherosclerotic, have been extensively studied and were revised by several authors.[4,5] These activities are usually attributed to polyphenols such as epicatechin, epigallocatechin, epicatechin-3-gallate, and especially epigallo-catechin-3-gallate, which are considered the most active catechins.[5] These and several other polyphenols are indeed extracted when green tea is brewed for consumption, as illustrated by Fig. 1, which shows in pictorial form the polyphenols that were identified and quantified as components of a hot water extract in a previous work of our group.[6] The data in Fig. 1 are not the result of exhaustive extractions. They reflect much more the contents that are likely to be present in the

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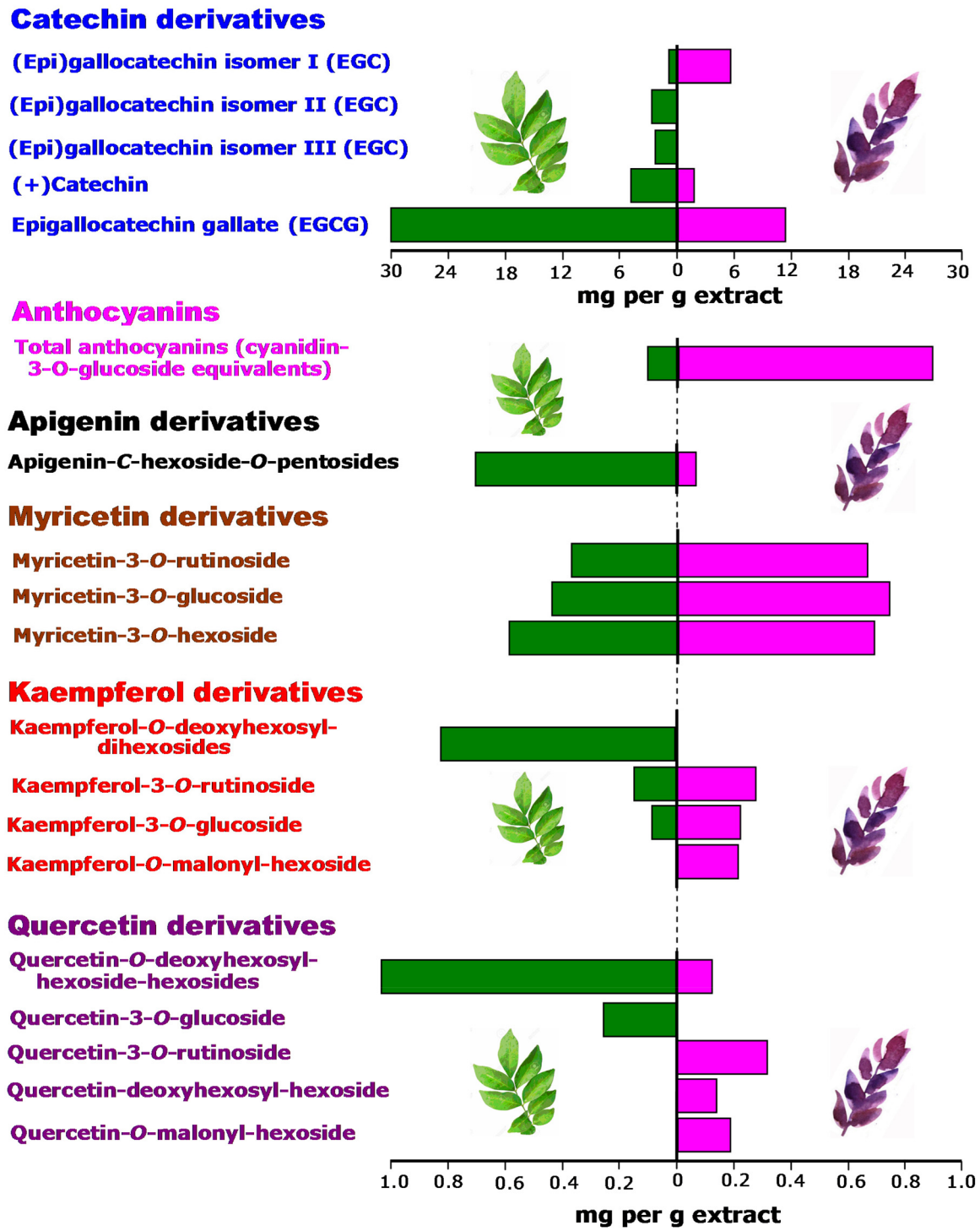

Fig. 1 Phenolic contents (non-anthocyanin and cyanin derivatives) of green and purple tea preparations according to a recent publication.[6]

beverage that is normally consumed by humans. Fig. 1 also shows the data obtained in the same study with purple tea.[6] The total anthocyanin content of the hot water purple tea extract greatly exceeds that of the corresponding green tea extract. The green tea preparation, on the other hand, considerably exceeds the corresponding purple tea preparation in terms of its content in non-anthocyanin polyphenolics, which were found to be equal to 44.8 and 23.0 mg g^{-1}, respectively.[6] However, this is basically a consequence of the much higher content of epigallocatechin gallate in the green tea preparation, which has been found to contain 30.2 mg g^{-1} whereas the purple tea extract contains only 11.6 mg g^{-1} (see Fig. 1). The much higher epigallocatechin gallate levels do not necessarily imply much higher biological activities for green tea

except for those resulting specifically from the action of the former compound. The actions of these two tea preparations on starch digestion is illustrative in this respect: although green tea inhibits starch digestion, the effects of purple tea were more pronounced, evidencing that epigallocatechin gallate was not the main compound responsible for the observed action.[6]

There are several claims that anthocyanins that are found in other plants of human consumption possess a series of health beneficial properties.[7–10] The relative richness of purple tea in anthocyanins, when compared to green tea, raises the question if the former is more active in biological events that are known to be especially sensitive to this class of compounds. A recent work in our laboratory, for example, emphasized the role of anthocyanins as possible inhibitors of triglyceride digestion. It was found that in mice, an extract of the *Myrciaria jaboticaba* peel containing anthocyanins was able to inhibit triglyceride absorption at doses starting at 1 mg kg^{-1} and reached 70% inhibition at the dose of 5 mg kg^{-1}.[11] These are very low doses in terms of a crude plant extract. Parallel experiments with pure cyanidin-3-*O*-glucoside, the most abundant anthocyanin in the *Myrciaria jaboticaba* peel extract, indicate that these compounds are certainly strong inhibitors of triglyceride absorption.[11] This observation leads to the hypothesis that purple tea preparations might also be active on triglyceride absorption, in part at least because of their contents in anthocyanin polyphenols. In consequence, the present work was planned mainly to investigate this question *via* both *in vitro* and *in vivo* experiments, the first ones consisting of lipase activity measurements and the latter ones of triglyceride tolerance tests in mice. Extracts closely simulating the mode of preparation of previous animal and human studies were used to facilitate comparison with previous investigations.[6,13,14] These measurements were preceded by the identification and quantification of the various anthocyanins present in the purple tea extracts that were used and by the measurement of a few basic properties such as antioxidant, anti-inflammatory, and cytotoxic activities. For comparison purposes, all experiments were done in parallel with similar green tea extracts.

Materials and methods

Materials

2,2-Diphenyl-1-picrylhydrazyl (DPPH), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (trolox), orlistat (tetrahydrolipstatin), thiobarbituric acid, sulforhodamine B, ellipticine, dexamethasone, and pancreatic lipase were acquired from Sigma-Aldrich, St Louis, MO, USA. The murine macrophage cell line (RAW 264.7) was purchased from the European Collection of Authenticated Cell Cultures (ECACC). The human tumor cell lines MCF-7 (breast adenocarcinoma), NCI-H460 (non-small cell lung cancer), HeLa (cervical carcinoma), and HepG2 (hepatocellular carcinoma) were acquired from the Leibniz-Institut DSMZ, Germany. All other chemicals were commercially available materials at the highest grade.

Animals

Fifty-days-old male *Swiss* mice (25 ± 2 g) were supplied by the Animal Breeding Center of the State University of Maringá (UEM) and maintained under standard animal house conditions at a temperature of 24 ± 2 °C and subject to a regulated 12 h light/dark cycle. The animals were housed in rodent steel cages (6–8 mice per cage) and fed with a standard mouse diet (Nuvilab®, Colombo, Brazil). After 3 days of acclimation, 15 h fasted animals were used for experiments. This study was performed in strict accordance with the Brazilian Council for the Control of Animal Experimentation (CONCEA; Federal law number 11794/2008) guidelines for the care and use of experimental animals and were approved by the Ethics Committee for Animal Use (CEUA) of UEM (protocol number 4155250919). Euthanasia was performed by injecting an overdose of anesthetic (150 mg kg^{-1} thiopental) intraperitonially, as recommended by the approved protocol.

Preparation of the aqueous tea extracts

Commercial green tea (*Camellia sinensis* var. *assamica*) was obtained from a local specialized store (Maringá, Brazil). Kenyan purple tea (purple tea) was ordered from Justea.com, Vancouver, Canada. The cultivar is called TRFK 306/1, originated from Kenya.[12] The extracts were prepared as described previously.[6,13] Grounded tea leaves (100 g) were boiled in distilled water (1000 mL) with subsequent stirring for 15 min at 70 °C (repeated 3 times). These extracts were filtered, frozen, and lyophilized under vacuum at −20 °C.

Analysis of anthocyanin phenolic compounds

A mixture of methanol/water (80 : 20, v/v) was used to dissolve the lyophilized extracts (10 mg mL^{-1}). Solubilization was followed by filtration through filter disks (0.22 μm). Chromatographic separation was performed in a HPLC system (Dionex Ultimate 3000 UPLC, Thermo Scientific, San Jose, CA, USA) coupled to a diode-array detector (DAD, using 280, 370, and 520 nm as the preferred wavelengths. Analysis was done mainly by a Linear Ion Trap (LTQ XL) mass spectrometer (MS, Thermo Finnigan, San Jose, CA, USA) coupled to a chromatographic system, which was equipped with an electrospray ionization (ESI) source. The anthocyanin phenolic compounds were separated using a Waters Spherisorb S3 ODS-2 C18 column (3 μm, 4.6 mm × 150 mm; Waters, Milford, MA, USA).[15,16] Identification was based on the comparisons of retention times and UV-Vis and mass spectra with those of standard compounds. If not possible, the data reported in the literature were applied to tentative identifications. In these cases, quantification was based on the calibration curves of the most similar standard. The results of the three analyses are given as means ± standard deviations (SD) and expressed in mg per g of the lyophilized extract.

Measurement of antioxidant activities

Five methods were used. Three of them were traditional *in vitro* assays, namely, 2,2-diphenyl-1-picrylhydrazyl (DPPH),

reducing power of iron ions (FRAP), and oxygen radical absorbance radical (ORAC) assays. In the other two, the oxidative hemolysis inhibition test (OxHLIA) and the inhibition of the production of thiobarbituric acid reactive substances (TBARS), biological systems were used. The evaluation of FRAP and ORAC was carried out as previously described,[17] the same being valid for the DPPH assay.[18] FRAP and ORAC data were expressed as μmol Trolox equivalents (TE) per mg of extract, whereas the DPPH assay results were expressed as IC_{50} values (concentrations displaying 50% antioxidant activity). The TBARS assay followed a methodology described elsewhere.[19] The ability of inhibiting the formation of thiobarbituric acid reactive substances (TBARS) was measured using the oxidizable substrates in porcine brain cell tissues. The outcomes were given as IC_{50} values (mg mL^{-1}) corresponding to the extract concentrations having 50% antioxidant activity. The oxidative haemolysis inhibition assay (OxHLIA) was carried out as described elsewhere.[19] IC_{50} values (μg mL^{-1}) were computed for a time period of 60 min. Thus, they mean the extract concentration that maintains 50% of the erythrocyte population intact for 60 min. Trolox was the positive control in both the assays.

Measurements of cytotoxicity and anti-inflammatory activity

The cytotoxicity of the extracts was assessed by means of the sulforhodamine B assay against four human tumor cell lines according to the protocol described previously.[20] The same assay was used to evaluate the hepatotoxicity against a non-tumor cell line (PLP2, porcine liver primary cells).[20] Ellipticine was the positive control. The results were expressed in GI_{50} values (μg mL^{-1}), meaning the extract concentrations causing 50% of cell growth inhibition.

Inhibition of the nitric oxide (NO) production by a lipopolysaccharide (LPS)-stimulated murine macrophage cell line (RAW 264.7) was used for estimating the potential anti-inflammatory activity of the extracts. The NO production was measured using a Griess Reagent System kit.[18] Incubations with dexamethasone were the positive controls, while the assays in the absence of LPS were taken as the negative controls. The effects of the extracts on the basal levels of NO production were also evaluated in incubations without LPS. The results were expressed as IC_{50} values (μg mL^{-1}).

In vitro pancreatic lipase assay

Porcine pancreatic lipase was assayed as its *p*-nitrophenyl-palmitate hydrolyzing activity.[21] Stock solutions of *p*-nitrophenyl-palmitate were prepared by dissolving it in isopropanol. The stock solution of the enzyme was prepared by dissolving 2 mg per mL in 100 mM Tris-HCl buffer (pH 8.2) and centrifuging at 2000*g*. The supernatant was the enzyme source. The final reaction mixture (2.5 mL) contained 100 mM Tris-HCl buffer (pH 8.2), varying concentrations of the substrate (in the range between 25 and 500 μM), enzyme, varying concentrations of tea extracts, and 20% isopropanol. For running the reaction, two protocols were used, the first one without previous incubation of the tea extracts with the enzyme and the second one with the pre-incubation of the enzyme with the extracts. In the first procedure, the tea extracts (100 μL) and the enzyme (100 μL) were added to 1800 μL of the pre-warmed reaction buffer at 37 °C, and the reaction was started by adding an isopropanol substrate solution (500 μL). In the second procedure, the tea extracts (100 μL) and the enzyme (100 μL) were added to 1800 μL of the buffer solution and after incubating the mixture at 37 °C for 10 min, the substrate (500 μL) was added for starting the reaction. The reaction was interrupted by immersing the reaction vessel into boiling water for 10 min. After cooling to room temperature, the reaction mixture was centrifuged at 1500*g* for 5 min. The absorbance of the supernatant at 410 nm was measured against a blank solution containing denatured enzyme.

Triglyceride tolerance

Triglyceride digestion *in vivo* was inferred from triglyceride tolerance tests, which were done as described previously.[11,21] The experimental animals (male Swiss mice, 25–35 g) were fasted (18 h). Seven groups (n = 3 per group) were utilized. The mice of group 1 received (by gavage) solely olive oil (5 mL kg^{-1}) intragastrically; these animals are mentioned as the controls. The animals of the second group received tap water and are mentioned as the negative controls, which were used for the determination of the basal line. Group 3 was the positive control, animals to which olive oil plus orlistat (50 mg kg^{-1}) was administrated. Groups 4, 5, and 6 were the mice that received, in addition to olive oil, various doses of the freeze-dried purple tea extract, more precisely, 100, 250, and 500 mg kg^{-1}, respectively. Group 7 finally received olive oil and 500 mg kg^{-1} green tea extract. The extracts and orlistat administrations preceded the olive oil administration. The plasma triglyceride levels were determined at 0, 90, 180, 270, and 360 min after olive oil administration in blood samples taken from the tail vein. The measurement of blood triglycerides was carried out by means of an AccutrendPlus® Roche triglyceride meter.[22]

Statistical and numerical analyses

Student's *t*-test was used when the difference between the two means was analyzed. Data sets composed of more than two means were subjected to univariate or multivariate variance analyses (ANOVA and MANOVA) according to the context, with *post-hoc* Newman–Keuls-testing. Significance was accepted when $p \leq 0.05$.

The Scientist software from MicroMath Scientific Software (Salt Lake City, UT) was utilized for fitting kinetic rate equations to the experimental initial rates of enzymatic activity by means of an iterative non-linear least-squares procedure. The decision about the most probable equation was based on the model selection criterion (MSC) and on the standard deviations of the optimized parameters. In the present work, the model with the largest MSC value was considered the most appropriate, provided that the estimated parameters were positive. When the MSC values differed by less than 5%, the model yielding the smallest standard deviations for the estimated parameters was considered the most appropriate one.

Results

Anthocyanin profile of Kenyan purple tea extracts and chemico-biological characteristics

Table 1 shows the content of each phenolic anthocyanin compound that was identified in the extracts. The compounds of this class were only found in the purple tea samples. Mild hot water was used in the extractions and the quantities (mg) per gram extract displayed in Table 1 represent the amounts that were effectively extracted. Three cyanidin derivatives and one peonidin derivative were found. All four compounds are rather complex structures. Two cyanidin derivatives contain at least two hexose moieties, namely, cyanidin-*O*-dihexosyl-pentoside and cyanidin-*O*-deoxyhexosyl-hexoside; notably, the first one contains a pentose unit. A third cyanidin derivative contains an acetylated hexose (cyanidin-*O*-acetyl-hexoside). The peonidin derivative is also relatively complex and possesses three hexose moieties in its structure.

The antioxidant activities of green and purple teas, determined by chemical (DPPH, FRAP, and ORAC) and cell-based assays (TBARS and OxHLIA) can be found in the ESI Table 1S.† [23–25] For the DPPH and FRAP assays, no significant difference was found for the extracts, while the ORAC assay showed a modestly higher antioxidant activity for purple tea. Concerning the cell-based assays, both green tea and purple tea showed similar antioxidant activities.

The antitumoral effects of the green and purple tea extracts, expressed as concentrations that caused 50% of the inhibition of cell growth (GI_{50}), can be found in ESI Table 2S.† The data show that green and purple tea extracts presented cytotoxic and anti-inflammatory activities significantly inferior to those of the positive controls ellipticine and dexamethasone, respectively. The values of GI_{50} for green tea and purple tea did not generally differ statistically, excepting the cases of MCF7 and HeLa cells. In relation to the anti-inflammatory activity, no significant difference was also observed between green and purple tea.

Effects on pancreatic lipase

Fig. 2 shows the concentration dependences of the effects of purple and green teas on the porcine pancreatic lipase *in vitro*. As described in the Materials and methods section, the assays were performed with or without the preincubation of the enzyme with the extract. Fig. 2A reveals that the pancreatic lipase was much more sensitive to purple tea when compared to green tea and that the sensitivity increased after preincubation with the extracts. At the purple tea extract concentration of 400 μg mL^{-1}, the inhibition was almost complete when the activity was measured after pre-incubation. The inhibition of the lipase caused by green tea, on the other hand, was considerably less pronounced than that of purple tea, but it also increased when preincubation was done. Under the latter condition, inhibition reached 40% at the concentration of 400 μg mL^{-1}.

Panel B of Fig. 2 shows the initial rates of the reaction catalyzed by the pancreatic lipase measured by simultaneously varying the concentrations of the substrate (*p*-nitrophenyl-palmitate) and of the purple tea extract. The reaction rates were measured after pre-incubation. The saturation curve for the substrate that was used showed the already reported phenomenon of substrate inhibition at high concentrations.[11] Each of the two concentrations of purple tea extract used compressed the saturation curve, and this effect was intensified when the concentration was increased from 50 to 200 μg mL^{-1}. For extracting the quantitative parameters from the concentration curves, attempts were made to fit different kinetic models, as outlined in the Materials and methods section. The equation that best described the data set in Fig. 1B is a function that accounts for substrate inhibition and for the formation of IEI and IESI complexes with quadratic [I] terms indicating multiple binding of the inhibitors.

$$v = \frac{V_{max}[S]}{K_M\left(1+\frac{[I]^2}{(\bar{K}_{i1})^2}\right)+[S]\left(1+\frac{[I]^2}{(\bar{K}_{i2})^2}\right)\left(1+\frac{[S]}{(K_{IS})}\right)} \quad (1)$$

K_{IS} is the substrate inhibition constant, $\bar{K}_{i1}$ reflects the average of the dissociation constants of the IE and IEI complexes, and $\bar{K}_{i2}$ represents the average of the dissociation constants of the IES and IESI complexes. K_M and V_{max} stand for the Michaelis–Menten constant and the maximal reaction rate, respectively. Fig. 1B allows to compare the experimental data (experimental

Table 1 Retention times (R_t), wavelengths of maximal absorption in the visible region (λ_{max}), mass spectral data, tentative identification, and quantification (mg g^{-1} of extract) of anthocyanin phenolic compounds present in the extracts of purple tea. Full experimental details are given in the Materials and methods section

Peak	R_t (min)	λ_{max} (nm)	Molecular ion $[H]^+$ (m/z)	MS^2 (m/z)	Tentative identification	Quantification (mg g^{-1} extract)
1	8.43	521	743	287 (100)	Cyanidin-*O*-dihexosyl-pentoside[a]	0.111 ± 0.004
2	11.37	513	787	301 (100)	Peonidin-*O*-deoxyhexosyl-hexuronosyl-hexoside[b]	0.48 ± 0.02
3	26.04	526	491	287 (100)	Cyanidin-*O*-acetyl-hexoside[a]	0.221 ± 0.004
4	28.41	523	595	449 (5), 287 (100)	Cyanidin-*O*-deoxyhexosyl-hexoside[a]	0.46 ± 0.02
					Total	1.27 ± 0.01

Quantitative analysis was performed using a 7-level calibration curves of each available standard constructed upon the UV signal. [a] Cyanidin-3-*O*-glucoside ($y = 105\,078x - 12\,437$; R^2: 0.9993; LOD = 0.28 μg mL^{-1}; LOQ = 0.84 μg mL^{-1}). [b] Peonidin-3-*O*-glucoside ($y = 122\,417x - 447\,974$, R^2 = 0.9965, LOD = 0.19 μg mL^{-1}; LOQ = 0.39 μg mL^{-1}).

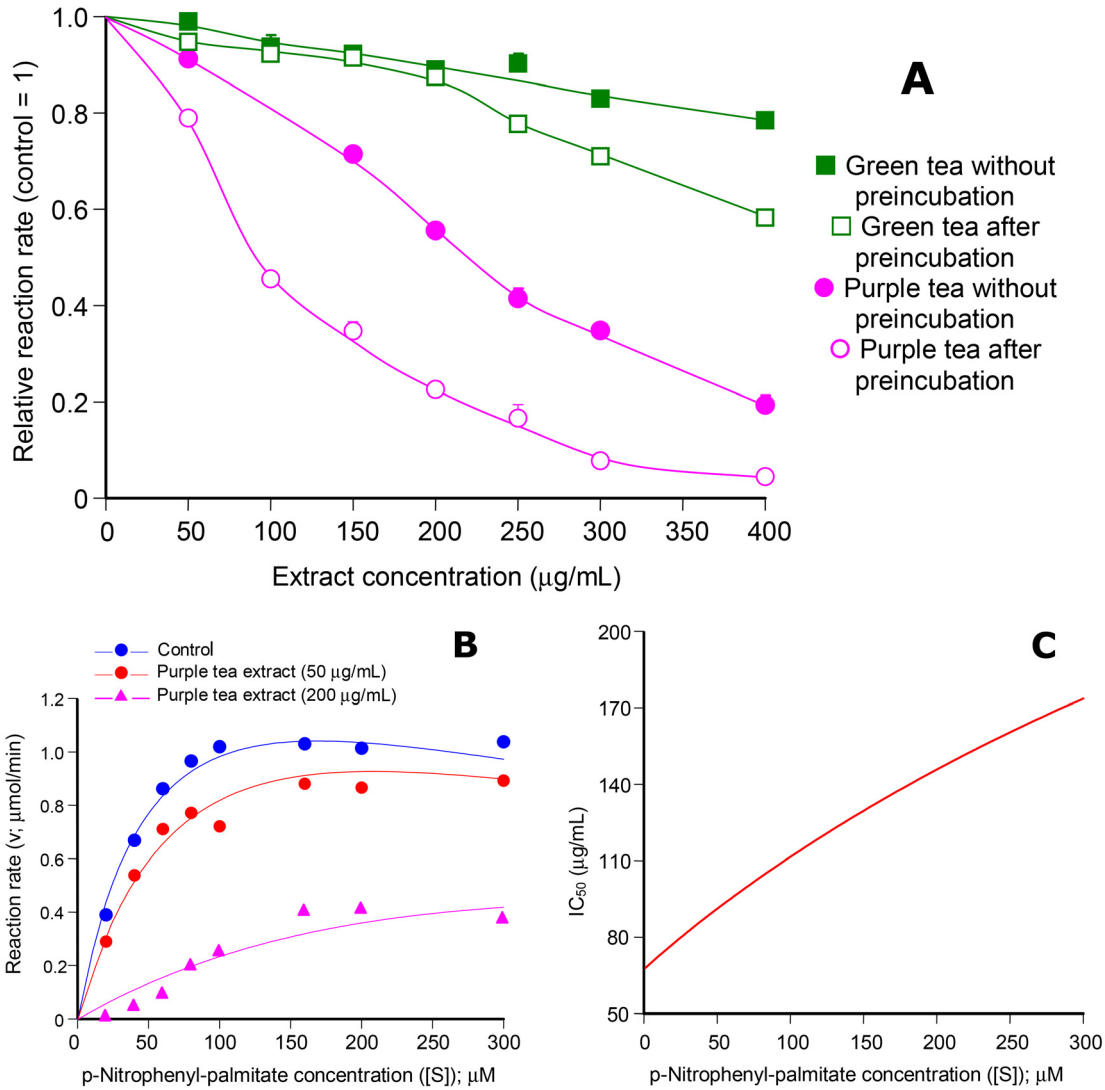

Fig. 2 Actions of purple and green tea extracts on the porcine pancreatic lipase *in vitro*. (A) Concentration dependences of the reaction rates on the extract concentrations. The reaction rates were measured with or without prior incubation of the enzyme with the extract, as indicated in the right side of the graph. Each data point is the mean of three determinations. Standard errors of the mean cannot be seen when smaller than the symbols. (B) Substrate saturation curves of the porcine pancreatic lipase at different purple tea extract concentrations. Each experimental point is the average of two determinations. The lines running through the experimental points were calculated using the optimized parameters obtained by fitting eqn (1) to the experimental data using a nonlinear least squares procedure. The values of the optimized parameters and goodness of fit indicators are: K_M, 61.17 ± 18.59 μM; V_{max}, 1.79 ± 0.30 μmol min^{-1}; K_{IS} = 468.65 ± 215.62 μM; $\bar{K}_{i1}$, 67.39 ± 33.0 μg mL^{-1}; $\bar{K}_{i2}$, 317.31 ± 324.86 μg mL^{-1}; sum of squared deviations, 0.0512; MSC, 3.54; correlation, 0.99. (C) Calculated IC_{50} values for the lipase inhibition by the purple tea extract as a function of substrate concentration. The IC_{50} values were computed through eqn (2) using the optimized parameters.

points) with the curves calculated using eqn (1) and the optimized parameters listed in the legend. Although there is a certain dispersion of the data, the calculated curves do not show systematic deviations from the experimental curves. It should be noted that the value of $\bar{K}_{i2}$ is much larger than $\bar{K}_{i1}$, denoting that the complexes with the free enzyme (IE, IEI, or higher) form more easily than the complexes with the enzyme–substrate complex. The competitive component, therefore, is more pronounced than the non-competitive one.

The advantage of fitting a rate equation to the data is that it allows to calculate the IC_{50} values (inhibitor concentrations causing 50% inhibition) for a given range of substrate concentration. For calculating this parameter, the following equation can be derived from eqn (1), assuming 50% inhibition.

$$IC_{50} = \left[\frac{K_M + [S] + \frac{[S]^2}{K_{IS}}}{\frac{K_M}{(\bar{K}_{i1})^2} + \frac{[S]}{(\bar{K}_{i2})^2} + \frac{[S]^2}{K_{IS}(\bar{K}_{i2})^2}}\right]^{1/2} \quad (2)$$

The various parameters are already defined above. In panel C of Fig. 2, the calculated IC_{50} values were represented as a

function of the substrate concentration. It is quite clear that the IC_{50} value increases when the substrate concentration is raised. This occurs largely because the competitive component predominates over the non-competitive component. Note that when [S] approaches zero, the IC_{50} value tends to a limiting value that corresponds to the $\tilde{K}_{i1}$ value (*i.e.*, 67.39 μg mL^{-1}).

Action on triglyceride absorption in mice

The results of the triglyceride tolerance experiments shown in Fig. 3 should allow to infer if triglyceride digestion also occurs *in vivo*.[11,21] In these experiments, the mean basal level of plasma triglycerides was equal to 184.8 ± 20.0 mg dL^{-1}.[11,26] The results in panel A reveal that the time courses of the plasma concentrations of triglycerides were clearly modified when purple tea was administered to the mice prior to the olive oil load. All curves obtained under the influence of purple tea were compressed in a dose-dependent manner in the direction of the baseline curve (when water was administered in place of olive oil). Panel B of Fig. 3 shows that this was also the effect of orlistat, the classical inhibitor of triglyceride digestion. Panel 2 also reveals that the effect of green tea, even at a high dose of 500 mg kg^{-1}, did not produce modifications that could be interpreted as an inhibition of triglyceride digestion. The latter assumption is corroborated by panel C, which shows the areas under the curves in panels A and C subtracted from the area under the base line, which is given by the curve obtained after water administration. It is generally accepted that these areas reflect with good approximation the net rate of the intestinal triglyceride absorption process.[11,21] The area under the curve obtained after green tea administration did

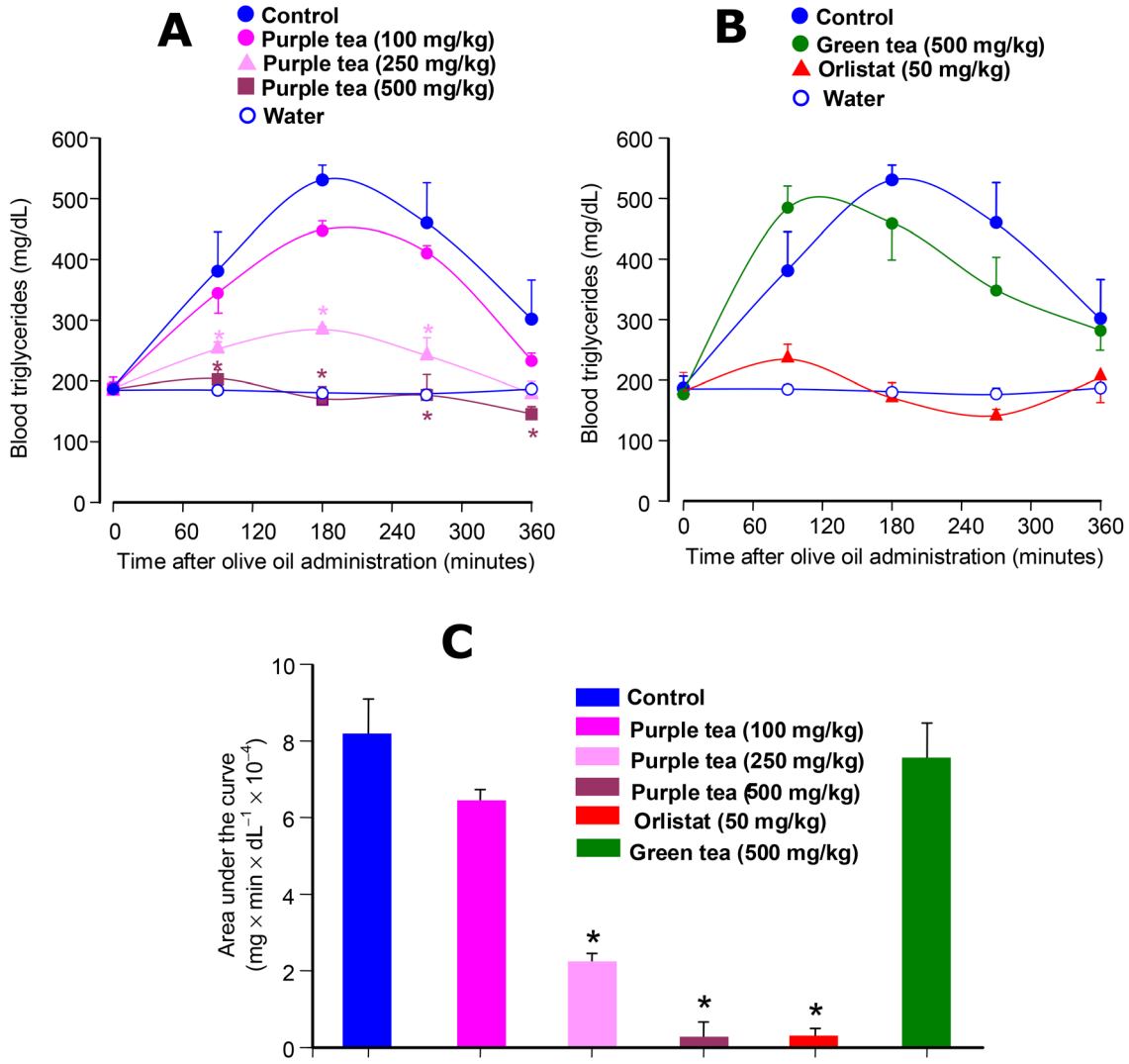

Fig. 3 Actions of purple and green tea extracts on triglyceride digestion *in vivo*. (A) Blood triglyceride concentration profiles after intragastric olive oil loads in mice and the effects of the previous administration of various purple tea extract doses. (B) Blood triglyceride concentration profiles after intragastric olive oil loads in mice and the effects of previous administration of orlistat and a single dose of green tea extract. (C) Areas under the curves in panels A and B after subtraction of the area under the basal curve obtained after tap water administration. Asterisks indicate statistical significance relative to the control curve in A (Newman–Keuls *post-hoc* testing after MANOVA) and relative to the control bar in C (Newman–Keuls *post-hoc* testing after ANOVA).

not differ from the control area. Different doses of purple tea, on the other hand, produced progressively smaller areas under the curves. The area computed for the 500 mg kg^{-1} dose was almost the same as that one found when orlistat was administered.

Discussion

In terms of their antioxidant, anti-inflammatory, and cytotoxic activities, both purple and green tea extracts were very similar. Cytotoxicity against normal cells (porcine liver primary cells) was considerably lower than that against tumor cells, an observation that is consistent with the traditional and widespread notion about the safety of the beverages brewed with *Camellia sinensis* leaves. Similar antioxidant and anti-inflammatory activities of both types of tea were found in spite of the higher levels of non-anthocyanin polyphenolic compounds of the green tea extract (Fig. 1).[6] Many different molecular species are involved, however, and qualitative differences probably play a significant role. It is also perfectly possible that the much higher content of the anthocyanins in the purple tea partially compensates its lower content in epigallocatechin gallate. The anthocyanin polyphenolics that were identified and quantified in our work, derivatives of cyanidin and peonidin, do not match those described in previous studies with other purple tea types.[2,3] However, in this respect, it must be remarked that the types and amounts of anthocyanin polyphenolics in the various purple tea clones that were examined so far also varied to considerable degrees.[2,3] Furthermore, the extraction procedure could also have influenced the results, as mild hot water extraction was done in our study and exhaustive methanolic extraction in previous reports.[2,3] Anthocyanins have important biological properties, which include antioxidant, anti-inflammatory, and anticarcinogenic activities.[27–29] The observation that both green and purple tea have similar antioxidant and anti-inflammatory activities allows to hypothesize that the latter is equally able to exert both activities *in vivo* as already demonstrated for green tea. The latter, for example, significantly diminishes oxidative stress and inflammation caused by adjuvant-induced arthritis in rats.[30] Alongside these and other similar actions of both tea varieties, however, there are two important ones in which the purple tea was revealed to display clear superiority: the inhibitory activities on the pancreatic lipase and on triglyceride digestion. The second effect can at least partly be the consequence of the first one, as will be discussed in the next paragraphs. It should be added, however, that the superior action of purple tea on fat digestion plainly parallels the superior action of this tea variety on starch digestion, as reported recently by our group.[6]

The kinetics of the inhibitory effects of the purple tea extract revealed a mixed or non-competitive parabolic mechanism of inhibition. The competitive component is much more pronounced, however, as revealed by the inhibition constants. From the view point of inhibition efficiency, this has the disadvantage that the inhibition degree diminishes when the substrate concentration is increased. If the inhibition of the lipase plays a significant role in the inhibition of triglyceride digestion, a greater load of fat will have a negative impact on the eventual inhibition caused by purple tea. This phenomenon is well demonstrated in panel C of Fig. 2, which shows how the IC_{50} value increases when the substrate concentration is increased. On the other hand, a parabolic type of inhibition means that inhibition is disproportionally enhanced when the inhibitor concentration is increased.[31] This is a phenomenon that obviously favors the inhibition of fat absorption as smaller increments of previous doses will be necessary to achieve new stipulated goals in terms of reductions in fat absorption. Another point deserving attention is synergism, a plausibility if one considers the great number of compounds that are probably involved. The existence of synergisms would be consistent with the observation that the inhibitory activity of purple tea is of the parabolic type, implying that at least two molecules in the extract can be bound by the enzyme simultaneously.[31]

The present report is not the first one in which the inhibitory effects of tea varieties on the pancreatic lipase are reported. The inhibitory effects on the pancreatic lipase have been reported for green, oolong, and white teas, for example.[32–34] Also, for purple tea, there is a previous report demonstrating the inhibition of the pancreatic lipase and triglyceride absorption by a hydro-ethanolic extract in mice.[35] A comparison of the latter study with our results, however, reveals an important difference in effectiveness. The ethanolic hydro-ethanolic extract used in this study caused a relatively modest inhibition of triglyceride absorption at the dose of 100 mg kg^{-1}, which was not further enhanced by raising the dose to 200 mg kg^{-1}.[35] Our experiments, on the contrary, revealed a well-defined dose-effect dependence, with almost complete inhibition at high doses. At least two reasons can be devised for this different behavior. The first one can be related to the mode of preparation, hot water extraction in our study and hydro-ethanolic extraction in the previous study.[35] It is also possible that the tea varieties that were used in both studies have different properties, although there is little experimental evidence that would allow to speculate about the possible reasons. It is worth mentioning, however, that hot water extraction is the usual way by which the beverage is prepared for current human consumption.

When compared to green tea, the superiority of the purple tea extract is valid for both the lipase inhibition *in vitro* and the triglyceride digestion *in vivo*. For the latter phenomenon, no significant effect was found with green tea even at a dose of 500 mg kg^{-1}. This dose of the purple tea extract almost entirely abolished triglyceride absorption in mice if one takes the action of orlistat as the maximal possible inhibition. The compounds that are involved in the inhibition of the pancreatic lipase might be several of the polyphenolics listed in Fig. 1. In fact, it is since long known that the catechins, epicatechins, and several other compounds found in the various tea varieties can inhibit the pancreatic lipase with various potencies.[30,32,33] It is very difficult, however, to deduce the relative participation

of each of the compounds listed in Fig. 1. The most abundant polyphenolic in both tea extracts, namely, epigallocatechin gallate (EGCG), is unlikely to be the most important participant. If it were so, the action of the green tea extract should be more pronounced as it contains three times more EGCG than the purple tea extract. Furthermore, *in vivo* experiments in the literature show that the action of EGCG on triglyceride absorption in rats is very poor even at doses of 50 and 100 mg kg^{-1}.[35,36] Actually even EGCG and other catechins extracted from purple tea were practically inactive on triglyceride digestion *in vivo*.[35] The 100 mg kg^{-1} dose is actually very high for a pure substance. For comparison, doses of 500 mg kg^{-1} green and purple tea extracts correspond to 15.1 and 5.8 mg kg^{-1} EGCG, respectively. Thus, the effects of the purple and green tea extracts result more likely from the combined actions of several inhibitors. The particular combination of the various molecular forms within the purple tea extract is evidently more favorable for producing inhibitory effects *in vitro* as well as *in vivo.* With reference to the molecular forms listed in Fig. 1, experimental data about the potency of the kaempferol, myricetin, and quercetin derivatives as inhibitors of the pancreatic lipase are scarce. There is a report, however, on the inhibitory action of kaempferol 3-*O*-rutinoside. According to this report, the compound would be a strong inhibitor of the lipase with an IC_{50} of 2.9 μM.[37] Fig. 1 reveals that this compound is more abundant in the purple tea than in the green tea preparation. Actually, kaempferol, myricetin, and quercetin rutinosides are all more abundant in purple tea than in green tea, a feature that might have some significance with respect to the inhibitory activity on the pancreatic lipase. Pure quercetin, on the other hand, has been demonstrated to affect both the pancreatic lipase and triglyceride absorption *in vivo*.[38] However, the total content in the quercetin derivatives of green tea exceeds that of purple tea by a factor of 1.7, although three of these derivatives are exclusively found in the latter (see Fig. 1).[6] If there are specific potency differences here, this cannot be inferred from the available data.

Although the inhibition of the pancreatic lipase by the non-anthocyanin phenolic compounds is likely to play an important role in the inhibition of fat absorption, the participation of the anthocyanin polyphenolics, which are much more abundant in purple tea, is equally probable. It is true that, in absolute terms, the contents of purple tea in these substances are relatively low. However, recent work has shown that anthocyanins can inhibit triglyceride absorption by two different mechanisms: (a) by inhibiting the pancreatic lipase, and (b) by impairing the transport of free fatty acids from the intestinal lumen into blood. Concerning the first mechanism, it has been found that most anthocyanin phenolics are inhibitors of the pancreatic lipase.[11,39] Their potencies vary greatly, the most potent ones having IC_{50} values between 40 and 50 μM.[40] This includes cyanidins as well as peonidins. These IC_{50} values do not indicate a very pronounced inhibitory potency and if one takes into account the absolute quantities that are present in purple tea, it seems that the participation of anthocyanins in the inhibition of the pancreatic lipase is not very pronounced. The second mechanism that was mentioned, *i.e.*, the direct inhibition of free fatty acid absorption, might be more important if one takes into account the low doses that are required. It was found in mice that cyanidin-3-*O*-glucoside starts to inhibit free oleate absorption at a dose of 0.2 mg kg^{-1}.[6] If one considers that a 500 mg kg^{-1} dose of the purple tea extract corresponds to the administration of at least 0.64 mg kg^{-1} of anthocyanins, it would not be a surprise if it turns out that this class of compounds could contribute in a highly significant way to the overall effects of purple tea on fat digestion that were detected in the present work. Evidently, this is a point pending on further investigations before a definitive conclusion can be reached.

A final comment must be made to differentiate the results obtained in our study with other investigations in which the actions of several varieties of *Camellia sinensis* on lipid metabolism were investigated. The experimental procedure used in the current study is adequate for quantifying the short-term effects on triglyceride absorption, especially those occurring as a consequence of the inhibition of the pancreatic lipase or fatty acid transport.[6] This phenomenon will evidently affect lipid metabolism in general, as revealed not only by lipid plasma levels but also by fat accumulation in the various tissues and other parameters. On the other hand, there are many mechanisms by which lipid metabolism can be affected and that will not necessarily involve triglyceride absorption. For the hydro-ethanolic purple tea extract, for example, it has been proposed that the inhibition of fat accumulation in mice and humans might be the consequence of an increased expression of the hepatic carnitine palmitoyl transferase when treatment was continued for a long period (17 days for mice).[35] Another study demonstrated that prolonged treatment (10 weeks) of mice with purple tea leaves ameliorates high-fat diet-induced obesity and metabolic disorders through the modulation of the gut microbiota.[41] Similar observations of medium- or long-term effects have been made with several other tea varieties, including green tea.[42–44] It seems, thus, logical to conclude that most types of tea may exert beneficial effects on lipid metabolism, depending on the dose and time of treatment.

Conclusion

The effects observed in the present study strongly suggest that the inhibition of the absorption of triglycerides may have an important contribution to the overall effects of purple tea on lipid metabolism. In this respect, the hot water purple tea extract is a much stronger inhibitor of the pancreatic lipase than the similar green tea extract. This inhibition reflects very strongly on triglyceride digestion *in vivo*, an activity that was significantly inhibited solely by the purple tea extract. This finding is comparable to the same effect reported previously for purple tea with respect to starch digestion. These observations, when combined, suggest that the regular consumption of purple tea can be useful in the control of both obesity and diabetes.

Data availability

Data sets supporting this article have been uploaded as part of the ESI.†

Author contributions

Tamires Barlati Vieira da Silva: Conceptualization, methodology, formal analysis, investigation, writing – original draft. Carla Pereira: Methodology, formal analysis, investigation, writing review & editing. Maria Inês Dias: Methodology, formal analysis, investigation. Filipa Mandim: Methodology, formal analysis, investigation. Marija Ivanov: Methodology, formal analysis. Marina Soković: Methodology, formal analysis, investigation. Lillian Barros: Writing review & editing. Isabel C. F. R. Ferreira: Writing review & editing. Flávio Augusto Vicente Seixas: Methodology, formal analysis, investigation. Adelar Bracht: Conceptualization, formal analysis, investigation, writing review & editing. Rosane Marina Peralta: Conceptualization, formal analysis, investigation, writing review & editing.

Conflicts of interest

The authors declare no conflict of interest.

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References

1 N. Sanlier, B. B. Gokcen and M. Altuğ, Tea consumption and disease correlations, *Trends Food Sci. Technol.*, 2018, **78**, 95–106, DOI: **10.1016/j.tifs.2018.05.026**.

2 L. C. Kerio, F. N. Wachira, J. K. Wanyoko and M. K. Rotich, Characterization of anthocyanins in Kenyan teas: extraction and identification, *Food Chem.*, 2012, **131**, 31–38, DOI: **10.1016/j.foodchem.2011.08.005**.

3 L. C. Kerio, F. N. Wachira, J. K. Wanyoko and M. K. Rotich, Total polyphenols, catechin profiles and antioxidant activity of tea products from purple leaf coloured tea cultivars, *Food Chem.*, 2013, **136**, 1405–1413, DOI: **10.1016/j.foodchem.2012.09.066**.

4 A. M. Teixeira and C. Sousa, A Review on the biological activity of *Camellia* species, *Molecules*, 2021, **26**, 2178–2203, DOI: **10.3390/molecules26082178**.

5 C. Musial, A. Kuban-Jankowska and M. Gorska-Ponikowska, Beneficial properties of green tea catechins, *Int. J. Mol. Sci.*, 2020, **21**, 1744–1175, DOI: **10.3390/ijms21051744**.

6 T. B. V. da Silva, P. A. Castilho, A. B. de Sá-Nakanishi, F. A. V. de Seixas, M. I. Dias, I. Barros, I. C. F. R. Ferreira, A. Bracht and R. M. Peralta, The inhibitory action of purple tea on in vivo starch digestion compared to other *Camellia sinensis* teas, *Food Res. Int.*, 2021, **150**, 110781, DOI: **10.1016/j.foodres.2021.110781**.

7 B. Alappat and J. Alappat, Anthocyanin pigments: beyond aesthetics, *Molecules*, 2020, **25**, 5500–5517, DOI: **10.3390/molecules25235500**.

8 C.-C. Lin, C.-P. Hsu, C. C. Chen, T. Z. Liao, C.-F. Chiu, P. J. Lien and Y. T. Shih, Anti-proliferation and radiation-sensitizing effect of an anthocyanidin-rich extract from purple-shoot tea on colon cancer cells, *J. Food Drug Anal.*, 2012, **20**, 328–331, DOI: **10.38212/2224-6614.2127**.

9 J. Shipp and E. Abdel-Al, Food applications and physiological effects of anthocyanins as functional food ingredient, *Open Food Sci. J.*, 2010, **4**, 7–22, DOI: **10.2174/1874256401004010007**.

10 A. C. Gonçalves, A. R. Nunes, A. Falcão, G. Alves and L. R. Silva, Dietary effects of anthocyanins in human health: a comprehensive review, *Pharmaceuticals*, 2021, **14**(7), 690–724, DOI: **10.3390/ph14070690**.

11 P. A. Castilho, L. Bracht, L. Barros, B. R. Albuquerque, M. I. Dias, I. C. F. R. Ferreira, J. F. Comar, T. B. V. da Silva, R. M. Peralta, A. B. de Sá-Nakanishi and A. Bracht, Effects of a *Myrciaria jaboticaba* peel extract on starch and triglyceride absorption and the role of cyanidin-3-*O*-glucoside, *Food Funct.*, 2021, **12**, 2644–2659, DOI: **10.1039/d0fo02927k**.

12 C. Kilel, A. K. Faranj, J. K. Wanyoko, N. Wachira and V. Mwingirwa, Green tea from purple leaf coloured tea clones in Kenya - their quality characteristics, *Food Chem.*, 2013, **141**(2), 769–775, DOI: **10.1016/j.foodchem.2013.03.051**.

13 K. Lochocka, J. Bajerska, A. Glapa, E. Fidler-Witon, J. K. Nowak, T. Szczapa, P. Grebowiec, A. Lisowska and J. Walkowiak, Green tea extract decreases starch digestion and absorption from a test meal in humans: a randomized, placebo-controlled crossover study, *Sci. Rep.*, 2015, **5**, 12015, DOI: **10.1038/srep12015**.

14 J. Bajerska, M. Wozniewicz, J. Jeszka, S. Drzymala-Czyz and J. Walkowiak, Green tea aqueus extract reduces visceral fat and decreases protein availability in rats fed with a high-fat diet, *Nutr. Res.*, 2011, **31**, 157–164, DOI: **10.1016/j.nutres.2011.01.005**.

15 S. M. F. Bessada, J. C. M. Barreira, L. Barros, I. C. F. R. Ferreira and M. B. P. P. Oliveira, Phenolic profile

and antioxidant activity of *Coleostephus myconis* (L.) Rchb. f.: An underexploited and highly disseminated species, *Ind. Crops Prod.*, 2016, **89**, 45–51, DOI: **10.1016/j.indcrop.2016.04.065**.

16 G. A. Gonçalves, A. A. Soares, R. C. G. Correa, L. Barros, C. W. I. Haminiuk, R. M. Peralta, I. C. F. R. Ferreira and A. Bracht, Merlot grape pomace hydroalcoholic extract improves the oxidative and inflammatory states of rats with adjuvant-induced arthritis, *J. Funct. Foods*, 2017, **33**, 408–418, DOI: **10.1016/j.jff.2017.04.009**.

17 E. A. Koehnlein, E. M. Koehnlein, R. C. G. Corrêa, V. S. Nishida, V. G. Correa, A. Bracht and R. M. Peralta, Analysis of a whole diet in terms of phenolic content and antioxidant capacity: effects of a simulated gastrointestinal digestion, *Int. J. Food Sci. Nutr.*, 2016, **67**, 614–623, DOI: **10.1080/09637486.2016.1186156**.

18 R. C. G. Corrêa, A. H. P. Souza, R. C. Calhelha, L. Barros, J. Glamoclija, M. Sokovic, R. M. Peralta, A. Bracht and I. C. F. R. Ferreira, Bioactive formulations prepared from fruiting bodies and submerged culture mycelia of the Brazilian edible mushroom *Pleurotus ostreatoroseus* Singer, *Food Funct.*, 2015, **6**, 2155–2164, DOI: **10.1039/C5FO00465A**.

19 L. Lockowandt, J. Pinela, C. L. Roriz, C. Pereira, R. M. V. Abreu, R. C. Calhelha, M. J. Alves, L. Barros, M. Bredol and I. C. F. R. Ferreira, Chemical features and bioactivities of cornflower (*Centaurea cyanus* L.) capitula: The blue flowers and the unexplored non-edible part, *Ind. Crops Prod.*, 2019, **128**, 496–503, DOI: **10.1016/j.indcrop.2018.11.059**.

20 R. M. Spréa, A. Fernandes, R. C. Calhelha, C. Pereira, T. C. S. P. Pires, M. J. Alves, C. C. Canan, L. Barros, J. S. Amaral and I. C. R. F. Ferreira, Chemical and bioactive characterization of the aromatic plant *Levisticum officinale* W.D.J. Koch: a comprehensive study, *Food Funct.*, 2020, **11**, 1292–1303, DOI: **10.1039/C9FO02841B**.

21 R. F. Oliveira, G. A. Gonçalves, F. D. Inácio, E. A. Koehnlein, C. G. M. Souza, A. Bracht and R. M. Peralta, Inhibition of pancreatic lipase and triacylglycerol intestinal absorption by a *pinhão* coat (*Araucaria angustifolia*) extract rich in condensed tannin, *Nutrients*, 2015, **7**, 5601–5614, DOI: **10.3390/nu7075242**.

22 C. Luley, G. Ronquist, W. Reuter, V. Paal, H. D. Gottschling, S. Westphal, G. L. King, S. J. L. Bakker, R. J. Heine and A. Hattemer, Point-of-care testing of triglycerides: valuation of the accutrend triglycerides system, *Clin. Chem.*, 2000, **46**, 287–291, DOI: **10.1093/clinchem/46.2.287**.

23 R. C. G. Corrêa, C. W. I. Haminiuk, L. Barros, M. I. Dias, R. C. Calhelha, C. G. Kato, V. G. Correa, R. M. Peralta and I. C. F. R. Ferreira, Stability and biological activity of Merlot (*Vitis vinifera*) grape pomace phytochemicals after simulated *in vitro* gastrointestinal digestion and colonic fermentation, *J. Funct. Foods*, 2017, **36**, 410–417, DOI: **10.1016/j.jff.2017.07.030**.

24 J. A. A. Garcia, R. C. G. Corrêa, L. Barros, C. Pereira, R. M. V. Abreu, M. J. Alves, R. C. Calhelha, A. Bracht, R. M. Peralta and I. C. F. R. Ferreira, Phytochemical profile and biological activities of "Ora-pro-nobis" leaves (*Pereskia aculeata* Miller), an underexploited superfood from the Brazilian Atlantic Forest, *Food Chem.*, 2019, **294**, 302–308, DOI: **10.1016/j.foodchem.2019.05.074**.

25 G. A. Gonçalves, R. C. G. Corrêa, L. Barros, M. I. Dias, R. C. Calhelha, V. G. Correa, A. Bracht, R. M. Peralta and I. C. F. R. Ferreira, Effects of *in vitro* gastrointestinal digestion and colonic fermentation on a rosemary (*Rosmarinus officinalis* L) extract rich in rosmarinic acid, *Food Chem.*, 2019, **271**, 393–400, DOI: **10.1016/j.foodchem.2018.07.132**.

26 M. S. Hussain, N. Jahan, M. M. O. Rashid, M. S. Hossain, U. Chen and N. R. Rahman, Antihyperlipidemic screening and plasma uric acid reducing potential of *Momordica charantia* seeds on Swiss albino mice model, *Heliyon*, 2019, **5**, e01739, DOI: **10.1016/j.heliyon.2019.e01739**.

27 R. Joshi, A. Rana, V. Kumar, D. Kumar, Y. S. Padwad, S. K. Yadav and A. Gulati, Anthocyanins enriched purple tea exhibits antioxidant, immunostimulatory and anticancer activities, *J. Food Sci. Technol.*, 2017, **54**, 1953–1963, DOI: **10.1007/s13197-017-2631-7**.

28 S. H. Lee, S. M. Park, S. M. Park, J. H. Park, D. Y. Shin, G. Y. Kim, C. H. Ryu, S. C. Shin, J. M. Jung, H. S. Kang, W. S. Lee and Y. H. Choi, Induction of apoptosis in human leukemia U937 cells by anthocyanins through down–regulation of Bcl–2 and activation of caspases, *Int. J. Oncol.*, 2009, **34**, 1077–1083, DOI: **10.3892/ijo_00000234**.

29 I. Elisia and D. D. Kitts, Anthocyanins inhibit peroxyl radical-induced apoptosis in Caco-2 cells, *Mol. Cell. Biochem.*, 2008, **312**(1–2), 139–145, DOI: **10.1007/s11010-008-9729-1**.

30 G. A. Gonçalves, A. B. de Sá-Nakanishi, M. M. N. Wendt, J. F. Comar, C. A. B. Amado, A. Bracht and R. M. Peralta, Green tea extract improves the oxidative state of the liver and brain in rats with adjuvant-induced arthritis, *Food Funct.*, 2015, **6**, 2701–2711, DOI: **10.1039/c5fo00548e**.

31 T. C. Chou and P. Talalay, A simple generalized equation for the analysis of multiple inhibitions of Michaelis-Menten kinetic systems, *J. Biol. Chem.*, 1977, **252**, 6438–6442, DOI: **10.1016/S0021-9258(17)39978-7**.

32 M. Nakai, Y. Fukui, S. Asami, Y. Toyida-Ono, T. Iwashita, H. Shibata, T. Mitsunaga, F. Hasimoto and Y. Kiso, Inhibitory effects of oolong tea polyphenols on pancreatic lipase *in vitro*, *J. Agric. Food Chem.*, 2005, **53**, 4593–4596, DOI: **10.1021/jf047814**.

33 L. Gulua, L. Nikolaishvili, M. Jgenti, T. Turmanidze and G. Dzneladze, Polyphenol content, anti-lipase and antioxidant activity of teas made in Georgia, *Ann. Agric. Sci.*, 2018, **16**, 357–361, DOI: **10.1016/j.aasci.2018.06.006**.

34 A. Gondoin, D. Grussu, D. Stewart and G. J. McDougall, White and green tea polyphenols inhibit pancreatic lipase *in vitro*, *Food Res. Int.*, 2010, **43**, 1537–1544, DOI: **10.1016/j.foodres.2010.04.029**.

35 H. Shimoda, S. Hitoe, S. Nakamura and H. Matsuda, Purple tea and its extract suppress diet-induced fat accumulation in mice and human subjects by inhibiting

fat absorption and enhancing hepatic carnitine palmitoyl transferase expression, *Int. J. Biomed. Sci.*, 2015, **11**, 67–75.

36 Y. F. Li, Y. Q. Chang, J. Deng, W. X. Li, J. Jian, J. S. Gao, X. Wan, H. Gao, H. Kurihara, P. H. Sun and R. R. He, Prediction and evaluation of the lipase inhibitory activities of tea polyphenols with 3D-QSAR models, *Sci. Rep.*, 2016, **6**, 34387, DOI: **10.1038/srep34387**.

37 S. Habtemariam, Antihyperlipidemic components of Cassia auriculata aerial parts: identification through in vitro studies, *Phytother. Res.*, 2013, **27**, 152–155.

38 J.-F. Zhou, W.-J. Wang, Z.-P. Yin, G.-D. Zheng, J.-G. Chen, J.-E. Li, L.-L. Cheng and Q.-F. Zhang, Quercetin is a promising pancreatic lipase inhibitor in reducing fat absorption in vivo, *Food Biosci.*, 2021, **43**, 101248, DOI: **10.1016/j.fbio.2021.101248**.

39 T. Buchholz and M. F. Melzig, Polyphenolic compounds as pancreatic lipase inhibitors, *Planta Med.*, 2015, **81**, 771–783, DOI: **10.1055/s-0035-1546173**.

40 L. Xie, J. Xie, Y. Xu and W. Chen, Discovery of anthocyanins from cranberry extract as pancreatic lipase inhibitors using a combined approach of ultrafiltration, molecular simulation, and spectroscopy, *Food Funct.*, 2020, **11**, 8527–8536, DOI: **10.1039/d0fo01262a**.

41 Y. C. Lin, H. F. Lu, J. C. Chen, H. C. Huang, Y. H. Chen, Y. S. Su, C. Y. Tung and C. Huang, Purple-leaf tea (*Camellia sinensis* L.) ameliorates high-fat diet induced obesity and metabolic disorder through the modulation of the gut microbiota in mice, *BMC Complementary Med. Ther.*, 2020, **20**, 376, DOI: **10.1186/s12906-020-03171-4**.

42 S. Huang, H. Chen, J. Teng, Z. Wu, L. Huang, B. Wei and N. Xia, Antihyperlipidemic effect and increased antioxidant enzyme levels of aqueous extracts from Liupao tea and green tea in vivo, *J. Food Sci.*, 2022, **87**, 4203–4220, DOI: **10.1111/1750-3841.16274**.

43 H. W. Jeong, J. H. Lee, J. K. Choi, C. S. Rha, J. D. Lee, J. Park and M. Park, Antihyper-triglyceridemia activities of naturally fermented green tea, Heukcha, extract through modulation of lipid metabolism in rats fed a high-fructose diet, *Food Sci. Biotechnol.*, 2021, **30**, 1581–1591, DOI: **10.1007/s10068-021-00992-y**.

44 Y. Shimamura, M. Yoda, H. Sakakibara, K. Matsunaga and S. Masuda, Pu-erh Tea suppresses diet-induced body fat accumulation in C57BL/6J mice by down-regulating SREBP-1c and related molecules, *Biosci. Biotechnol. Biochem.*, 2013, **77**, 1455–1460, DOI: **10.1271/bbb.130097**. Epub 2013 Jul 7.