Characterizing the Phenolic Composition and Exploring the Antioxidant, Anti-Cancer, and Anti-Inflammatory Potential of Medicinal Plant Infusions

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Abstract

Natural products and their derivatives have played important roles in various fields, including nutrition, medicine and cosmetics. In this study, different formulated plant infusions, namely Purifying Body (PB), Boost Training (BT), Calm Night I Spirit (CN), and Boost Energy (BE) were analyzed regarding their phenolic composition and biological properties such as antioxidant, anti-inflammatory, and its influence on a different type of tumoral and non-tumoral cells. Nineteen phenolic compounds across six groups were identified, being cinnamic acid the most prevalent in all infusions. BE infusions exhibited the highest total phenolic content (5 g GAE/L), while BT had the lowest (2 g GAE/L). BE demonstrated superior antioxidant activity, correlating with its high phenolic content. The impact of plant infusions on cell metabolic activity revealing a concentration-dependent behaviour for three used cell lines [human embryonic kidney (HEK293t), normal mouse fibroblast (L929), and human colorectal adenocarcinoma (Caco-2)]. IC_{50} values indicated low toxicity on non-tumoral cells and higher inhibition on the metabolic activity of colon cancer Caco-2 cells. These effects can be due to the induction of cell cycle arrest and apoptosis. All plant infusions significantly prevented reactive oxygen species formation (oxidative stress regulation), and present anti-inflammatory capacity with a decrease of pro-inflammatory (IL-1β) and an increase of anti-inflammatory (IL-10) cytokines.

Overall, the formulated infusions have antioxidant and anti-inflammatory properties and a notable impact on colon cancer cells. This study revealed the potential of the tested infusions in various medical applications, such as preventive care, as well as in the nutraceutical field.

Keywords: Medicinal plants; Polyphenols; Cellular studies; Antioxidant action; Anticancer; Anti-inflammatory

Introduction

According to the Global Cancer Observatory (GLOBOCAN) estimates from the International Agency for Research on Cancer (IARC), cancer is one of the major causes of death worldwide, accounting for 9.74 million deaths in 2022, constituting approximately one in six total deaths [1]. The 2022 report highlights that lung cancer is the most prevalent in terms of the number of new cases (2.48 million cases, representing the 12.4% of total cases), followed by breast cancer (2.29 million cases, 11.5%), colon and rectum cancer (1.93 million cases, 9.6%), prostate cancer (1.47 million cases, 7.3%), stomach cancer (0.97 million cases, 4.8%), and liver (0.87 million cases, 4.3%). Despite significant advancements in understanding cancer biology in recent years, statistics still demonstrate that the number of deaths will continue to grow over the next years [2].

The well-established reality is that conventional cancer treatments, such as chemotherapy or radiotherapy, are associated with several side effects that significantly impact the quality of life for patients. Consequently, there is an ongoing necessity to explore and develop superior and more effective anti-cancer therapies, especially those with fewer associated side effects [3].

Throughout human civilization, plants and their derivatives (natural products) have played crucial roles in various domains, including medicinal, nutrition, and cosmetics [4]. Recognized as a rich source of bioactive compounds, plant natural products have demonstrated health-promoting potential in the prevention of non-communicable diseases, including cancer, cardiovascular illnesses, and metabolic disorders. Plant secondary metabolites, particularly phenolic compounds (identified more than 8000 molecules in nature), possess structural complexity that include phenolic acids, flavonoids, tannins, stilbenes and other phenols, which contribute to the therapeutic potential of many medicinal herbs. These bioactive molecules exhibit a broad spectrum of bioactivities [5,6], such as antioxidant, anti-inflammatory, and anticancer effects [7].

Phenolic compounds' remarkable anti-cancer efficacy can be attributed to their antioxidant activity, which is the ability to inhibit free radicals' formation and interfere with molecular targets and signaling pathways [8]. These pathways are closely linked to many important processes, such as cell survival, cell proliferation, differentiation, cell apoptosis, cell migration, angiogenesis regulation, hormonal activity, and immune responses [9–12].

79 The anti-inflammatory capacity is related to the immune response against bacteria, trauma,
80 toxins, heat, excessive exercise, and other causes. However, prolonged inflammation can lead to
81 chronic diseases such as cancer [13,14]. In this inflammatory response, the inflammatory
82 pathways are activated and markers are released to the blood. Some studies have demonstrated
83 the ability of phytochemicals like phenolics from several bioresources to inhibit pro-inflammatory
84 cytokines such as IL-1, IL-6, and TNF-α [13,15–18]. Research studies described that plant-
85 derived phytochemicals, like phenolics, contribute to maintaining protein homeostasis in the
86 cancer process [13]. Other investigations, including clinical trials, have proved the ability of
87 phytochemicals to reduce chemotherapy drugs' side effects as well as enhance their
88 effectiveness [13,19].
89 Considering the relevant data, herbal infusions, *i.e.* aqueous extracts prepared by steeping plant
90 materials in hot water, are a traditional and accessible means of delivering these phenolic
91 compounds. Due to their ease of preparation and cultural relevance, infusions are commonly used
92 in complementary medicine to support health and prevent chronic diseases.
93 historically, the core structures of plants' secondary metabolites can be used as templates for
94 drug design and the synthesis of new substances for the treatment of several diseases [16,20]. It
95 is estimated that approximately 25% of all approved anticancer drugs over the last 40 years were
96 related to natural products. For example, vincristine, irinotecan, etoposide and paclitaxel are
97 examples of plant-derived compounds employed in cancer treatment; dactinomycin, bleomycin
98 and doxorubicin are anticancer drugs derived from a microorganism; and trabectedin is a marine-
99 derived anticancer agent isolated from *Caribbean tunicate* and *Ecteinascidia turbinate* [21].
100 In the last 10 years, more than 107,567 research papers have been published describing the
101 effect of natural products on cancer, most of them demonstrating their anticancer activity *in vitro*
102 and *in vivo* [22–27]. Although, the exact mechanism of phytochemicals is not fully understood.
103 Although medicinal plants have long been recognized for their health-promoting properties,
104 comprehensive comparative studies on formulated plant infusions—especially in terms of their
105 phenolic profiles and multifunctional bioactivities—remain limited. In particular, there is a lack of
106 integrated analysis assessing the antioxidant, anticancer, and anti-inflammatory potential of
107 standardized multi-herbal infusions, which are increasingly used in traditional and functional
108 medicine. This study aims to fill this gap by characterizing the phenolic composition of four

carefully formulated medicinal plant infusions and evaluating their bioactive potential using *in vitro* assays. We hypothesize that these formulations contain synergistic combinations of phenolic compounds that contribute to significant therapeutic effects. The results will enhance scientific understanding of the bioefficacy of complex plant-based infusions and support their development as natural sources of therapeutic agents.

1. Materials and methods

2.1. Plant material

The medicinal plants were provided by the company Chá hunos®, comércio de plantas medicinais Lda. (Grijó, Portugal) and the infusions were formulated taking into account the Portuguese Pharmacopeia VII [28] by the company Stefitea® (Esposende, Portugal). The plant composition of the four formulated infusions, Purifying Body (PB), Boost Training (BT), Calm Night I Spirit (CN) and Boost Energy (BE) is presented in Table 1.

The herbal infusions were prepared by steeping 2 grams of dried formulated plant material (Table 1) in 100 mL of hot water (approximately 95 °C) for 10 minutes under covered conditions to minimize the loss of volatile compounds.

Table 1.

2.2. Phenolic content and identification

2.2.1. Total phenolic compounds' content

The TPC of all the samples was determined using the Folin-Ciocalteu's method, adapted to a 96-well microplate, as described by Jesus *et al.* [29]. Specifically, 10 µL of each infusion was mixed with 60 µL of sodium carbonate solution (7.5%, w/v), 15 µL of Folin–Ciocalteu reagent (Sigma-Aldrich, St. Louis, MO, USA), and 200 µL of distilled water in a microplate well. The mixture was then incubated at 60 °C for 5 minutes, and the absorbance was measured at 700 nm using a UV–Vis spectrophotometer (Synergy HT, BioTek), with a blank sample (water) as the reference. TPC was estimated using gallic acid (GA) as standard (R^2 = 0.998) and the results were expressed as g GA equivalents (GAE) per liter of infusion (g GAE/L).

2.2.2. Identification and quantification of individual phenolic compounds by UHPLC

Identification and quantification analysis of the phenolic compounds present in the plant infusions were performed by Shimatzu Nexpera X2 UHPLC chromatograph equipped with Diode Array Detector (Shimadzu, SPD-M20A), as previously described by Ferreira-Santos *et al*. [30]. The separation was performed on a reversed-phase Acquity UPLC BEH C18 column (2.1 mm × 100 mm, 1.7 µm particle size; from Waters) at 40 °C. The injection volume of 10 µL and a flow rate was 0.4 mL/min. The solvents used were water/formic acid (0.1%) as solvent A and acetonitrile as solvent B, the elution gradient used for solvent B was: from 0.0 to 5.5 min eluent B at 5%, from 5.5 to 17 min a linear increase to 60%, from 17.0 to 18.5 min a linear increase to 100%, then column equilibration from 18.5 to 30.0 min at 5%.

Phenolic compounds were identified by comparing their UV spectra and retention times with that of corresponding standards. Quantification was carried out using calibration curves for each compound analyzed using concentrations between 250 and 2.5 mg/L ($R^2 > 0.989$), and take into account the limit of detection (LOD) and limit of quantification (LOQ) for each compound. The wavelength was adjusted for each target compound (209–370 nm) [31]. The values of individual phenolic compounds were expressed in milligrams per liter of plant infusion (mg/L). All standards were of analytical grade (purity < 97%) and procured from Sigma Aldrich (St. Louis, MO, USA).

2.3. Antioxidant activity

Three different methods were employed to assess different mechanisms of the antioxidant action: DPPH and ABTS as radical scavenging capacity, and FRAP as reducing antioxidant capacity. The antioxidant activity was determined using the methods described by Ferreira-Santos *et al.* [30]. 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) was used as a standard for DPPH and ABTS methods, and ferrous sulphate for FRAP method. The antioxidant activity values were expressed as millimoles of standards equivalent (TE) per liter of plant infusion (mmol TE/L).

2.4. Cell viability

In vitro cell metabolic activity was assessed in human embryonic kidney (HEK293t – ATCC CRL-11268), normal mouse fibroblast (L929 – ATCC CCL-185), and human colorectal

adenocarcinoma (Caco-2 – ATCC HTB-37™); these cell lines were kindly provided by Andreia Gomes (Department of Biology, University of Minho). Cells were grown in supplemented Dulbecco's Modified Eagle Medium (DMEM) following the protocol reported by Ferreira-Santos *et al*. [32]. The cells ($1x10^5$ cells per well) were incubated for 48h with supplemented DMEM or plant infusion extracts in a concentration between 62 and 2000 µg/mL, using water as the vehicle for extract dilution. Then, cell viability metabolic activity was determined by the resazurin method [32]. The percentage of cell viability metabolic activity was calculated by relating the sample values to untreated controls. IC_{50} values were calculated by a dose-response curve using GraphPad software.

2.5. Apoptosis and cell cycle analysis

Human Caco-2 cells were exposed to IC_{50} concentration of the four different plant infusions for 48 h; then they were collected and stained with Annexin V-FITC. Propidium iodide (PI) stained cells were analyzed for DNA content and the percentage of cells in cycle phases was determined following the protocol of Sanchez-de-Diego *et al.* [33]. To determine basal levels of apoptosis, necrosis and cell death, untreated cells were used as negative control.

2.6. Measurement of intracellular ROS levels

A commercial kit (ab 113851, DCFDA/H2DCFDA - Cellular ROS Assay Kit by Abcam plc®, Cambridge, UK) that uses tert-butyl hydrogen peroxide (TBHP) as a ROS inducer was used to determine the intracellular ROS levels of Caco-2 cells exposed to the IC_{50} concentration of the different plant infusions for 12 hours. This assay was carried out according to the manufacturer's instructions and previously reported by us [34] .

2.7. Anti-inflammatory activity in macrophage

The macrophage cell line (J774A.1, ATCC TIB-67 ™) was used to assess cell viability and cytokine secretion following incubation with the extracts. The cell line was cultured in DMEM high glucose, supplemented with 10% FBS, 2 mM glutamine, 1 mM sodium pyruvate, and 25 mM HEPES buffer. The cultures were maintained in tissue culture flasks (Nagle Nunc, Int., Hereford,

198 UK) with a humidified atmosphere containing 5% CO2 at 37 °C (Binder CB150; Tuttlingen,
199 Germany).
200 After confluent growth, macrophage cells were washed with a fresh medium and recovered by
201 scrapping. Viable cells were counted by Trypan blue exclusion in the hemocytometer and
202 resuspended in DMEM to a final concentration of 1×10^6 cells/mL. A volume of 300 µL of the
203 macrophage suspension was then cultured in 48-well tissue culture plates.
204 Cells were incubated overnight with or without 1 µg/mL of lipopolysaccharides (LPS, Merk), to
205 establish inflammation and non-inflammation models, respectively. Following incubation, cells
206 were washed with fresh medium, and treated with 2500 µg/mL of plant infusions (with or without
207 the LPS at 1 µg/mL) for 24h. The concentration of plant infusions was selected based on
208 preliminary results (data not shown), and no significant cell viability reduction by MTT assay. After
209 treatment, the supernatants were collected and stored at −20 °C for cytokine quantification.
210 The metabolic viability of the cells was determined using the MTT assay [35]. Formazan crystals
211 formed were dissolved in a DMSO:ethanol (1:1) solution, and absorbance was measured at 570
212 nm. Controls included cells incubated only with DMEM, with the extracts' solvent (PBS), and 1
213 µg/mL of LPS.

214 **2.7.1. Cytokine quantification**

215 The concentrations of Tumour Necrosis Factor (TNF)-α, interleukin (IL)-1β, IL-6, IL-10,
216 and Transforming growth factor (TGF)-β1 in cell culture supernatants were measured using the
217 corresponding Mouse Uncoated ELISA kit (Invitrogen), following the manufacturer's instructions.
218

219 **2.8. Statistical analyses**

220 The statistical analysis was conducted using the Graph Pad Prism 5 Software (Graphpad
221 Software in San Diego, CA, USA). The data are presented as the mean ± standard deviation (SD)
222 obtained from at least three tests for each experiment. The study employed one- or two-way
223 analysis of variance (ANOVA) followed by the Tukey test to evaluate variations across the
224 different groups. Values were deemed statistically significant for p-values less than 0.05
225 (confidence level of 95%).
226

227 **3. Results and discussion**

Table S1 presents a total of 23 medicinal plants used in the formulation of infusions analyzed in this work, highlighting their health benefits as reported in the scientific literature. Among the most commonly attributed effects are antioxidant, anti-inflammatory, antimicrobial, hepatoprotective, neuroprotective, and antidiabetic properties. Some species have also shown potential anticancer, anxiolytic, and antidepressant activities, as well as benefits for skin regeneration and cardiovascular health. These therapeutic effects are generally associated with the presence of bioactive compounds such as flavonoids, alkaloids, polyphenols, and terpenoids found in different parts of the analyzed plants.

The four formulated infusions – *Purifying Body*, *Boost Training*, *Calm Night I Spirit*, and *Boost Energy* – combine various medicinal plants known for their proven therapeutic effects. The *Purifying Body* infusion, composed of plants such as *Cichorium intybus*, *Betula alba*, *Peumus boldus*, *Cochlospermum angolensis*, *Taraxacum officinale*, and *Cassia angustifolia*, stands out for its hepatoprotective, antioxidant, diuretic, and detoxifying properties**,** promoting detoxification and the proper functioning of the liver and kidneys. The *Boost Training* infusion includes plants such as *Illicium verum*, *Centella asiatica*, *Camellia sinensis*, *Phaseolus vulgaris*, *Paullinia cupana*, and *Calluna vulgaris*, known for **their** anti-inflammatory, adaptogenic, neuroprotective, and energizing effects**,** making them especially suitable for physical and mental support during training activities. The *Calm Night I Spirit* infusion features plants such as *Foeniculum vulgare*, *Lavandula angustifolia*, *Lippia triphylla*, *Jasminum grandiflorum*, *Passiflora incarnata*, and *Verbena officinalis*, with calming, anxiolytic, antidepressant, and sleep-regulating activities**,** promoting relaxation and emotional well-being. Finally, the *Boost Energy* infusion, based on *Rosmarinus officinalis*, *Calendula officinalis*, *Equisetum arvense*, *Camellia sinensis*, *Ginkgo biloba*, and *Eleutherococcus senticosus*, offers stimulating, antioxidant, and cognitive benefits**,** contributing to increased vitality, focus, and mental performance.

3.1. Phenolic content and antioxidant activity

The TPC and antioxidant activity (DPPH, ABTS and FRAP assays) values obtained for the plant infusions are presented in Figure 1. TPC and ABTS assays exhibit no significant differences between the plant infusions. However, DPPH and FRAP assays have significant differences between the samples.

TPC, an essential parameter for evaluating the total antioxidant capacity of plants, was determined using the Folin-Ciocalteu method. This method is based on the electron transfer from phenolic compounds to the Folin-Ciocalteu reagent in alkaline media [36]. Our results show that TPC ranged from 2 to 5 g GAE/L (as shown in Figure 1). The BE plant infusions exhibited the highest TPC values, approximately 5 g GAE/L, while the lowest content was found in the BT plant infusions, registering 2 g GAE/L.

The antioxidant activity, a mechanism crucial for preventing oxidative stress-related diseases, relies on the composition and structure of biocompounds, particularly phenolic acids and flavonoids. Their ability to neutralize free radicals, acting as chelators and scavengers, contributes to their antioxidant activity [37].

Comparing the results of the three antioxidant activity assays performed (DPPH, ABTS and FRAP), BE plant infusion presented the highest antioxidant activity while the BT plant infusion showed the lowest values. The ABTS method compares the capacity of antioxidants to degrade ABTS into Trolox [37]. The results show that BE plant infusion has the highest radical scavenging activity, while CN plant infusion has the lowest value. These results show that the plant infusions have similar values, however, the BE plant infusion values are slightly higher than the other plant infusions. FRAP method is based on the ability of samples to donate electrons to reduce a Fe(III)-TPTZ complex to a blue Fe(II)-TPTZ complex [38]. The FRAP assay proved the potent reducing power of the BE plant infusion, which had the highest value of FRAP (19 mmol Fe(II)/L). The other plant infusions also have considerable values, however, not as high as the BE.

The antioxidant activity results are in accordance with TPC and individual phenolic compounds. The fact that the plant infusion with the most antioxidant activity is the one with the highest content of phenolic compounds leads to the conclusion that these compounds provide its antioxidant capacity. Similar results were observed in the literature [39], where it was shown that TPC is related with the phenolic content. Even though, it has been shown in 223 medicinal plants that phenolics components may not be responsible for the reducing oxidant ability, due to a weak correlation observed between the DPPH value and the FRAP [39]. Similarly, how results do not show a correlation between DPPH and TPC.

Figure 1.

3.2. Identification and quantification of individual phenolic compounds by UHPLC

To understand the results obtained regarding the chemical oxidant activity the plant infusions obtained were subjected to a comprehensive characterization. Chromatographic analysis (UHPLC) allowed the identification and quantification of individual compounds (Table 2). Nineteen phenolic compounds belonging to 6 different groups were identified according to the corresponding standards: hydroxybenzoic acid, hydroxycinnamic acid, flavanol, flavonol, flavanone and stilbene.

Cinnamic acid was present in all plant´s infusions at high concentrations, ranging from 176.12 ± 26 mg/L in the BT plant infusion to 1900.4 ± 100 mg/L in the BE plant infusion. Vanillic acid has only been identified and quantified in BE plant infusions, even though at a low concentration (0.34 ± 0.03 mg/L). Catechin was not identified in the PB plant infusion, and the CN plant infusion presented the highest concentration (175.42 ± 9.4 mg/L). Residual concentrations of catechin were found in the BT plant infusion (3.68 ± 0.1 mg/L) and at the BE plant infusion (13.45 ± 1.5 mg/L). Epicatechin was absent in BT and CN plant infusions, but identified in PB and BE plant infusions with 9.04 ± 0.3 and 7.48 ± 0.1 mg/L, respectively. Gallic acid was not identified in PB, while other studied plant infusions presented similar concentrations ranging from 22.54 ± 1.1 to 29.69 ± 1.2 mg/L. PB, BT and BE plant infusions present 2,5-dihydroxybenzoic acid (11.40 to 12.71 ± 0.2 mg/L), except CN plant infusions. Kaempferol was identified in BT, CN and BE plant infusion, however in low concentration (Table 2). Quercetin is present in all plant infusions, although the BT plant infusion has the lowest concentration.

The remaining phenolic compounds identified were consistently found in all four plant infusions, with infusions of PB and BE plants exhibiting the highest concentrations of almost all compounds. The detailed analysis of the chemical composition of the plant infusions allows us to state that these natural infusions are rich in phenolic acids and flavonoids.

As can be seen in Table 2 the BE presents the highest concentrations in phenolic compounds, which is in accordance with the results obtained regarding the chemical anti-oxidant activity. Moreover, an interesting result was observed regarding the high concentration of cinnamic acid. Cinnamic acid is known for its beneficial effects in terms of overall health, particularly regarding complications involved with diabetes, like diabetic cardiomyopathy [40,41]. Moreover, there are studies reporting the anti-inflammatory capacity of the cinnamic acid as well as, its involvement

in reducing oxidative stress [42]. Rutin and hesperidin are also known phenolic compounds with important beneficial effects, as antioxidants agents. A study using neuronal cells, as shown that rutin is an efficient free radical scavenger in a concentration-dependent manner with the ability to overcome oxidative and nitrosative stress [43]. Additionally, the authors have shown that rutin improves antioxidant enzymes and can reduce pro-inflammatory cytokines [43]. Regarding, hesperidin it has been shown that it has a significant number of health benefits, including antioxidant, antibacterial, antimicrobial, anti-inflammatory and anticarcinogenic properties [44]. Therefore, the antioxidant ability of BE may be related to the presence of high concentrations of cinnamic acid, rutin and hesperidin.

Table 2.

3.3. Cell viability

Even though the previous results are promising in terms of chemical anti-oxidant it was important to assess its behavior when in contact with cancerous and non-cancerous cells. So, infusions were placed in contact with different cell lines, one normal – HEK293t, one derived from cancer tissue – Caco-2, and one normal mouse cell line (L929). Their interactions were evaluated in terms of their ability to metabolize resazurin into resorufin, which is correlated with cellular metabolic activity and cell viability [45].

The cell lines chosen for this assay are aligned with the established practices to assess the potential toxicity of plant extracts and isolated compounds [32,46].

In this experiment, cells were exposed to different concentrations of each plant infusion (ranging from 0 - 2000 µg/mL). The results revealed a concentration-dependent decrease in cell viability in all three cell lines. However, notable differences were observed in the behaviour of Caco-2 compared to HEK293t and L929 cells. The exposure of Caco-2 cells to lower concentrations of the plant infusions (62 and 125 µg/mL) led to a decrease in cell metabolic activity, while the same concentrations of plant infusions induced an increase in metabolic activity in HEK239t and L929 cells, for almost all the tested plant infusions. This differential impact on cell viability has been previously documented for green tea in various human colorectal cancer cells, including SGC-7901, SW480, SW837, HCT116, HT29, K562, MDA-MB 231, and MCF7, when compared to

normal human colon cells [47–50]. This finding suggests a potential selective impact on cancer cells, sparing healthy cells. Nevertheless, it is crucial to acknowledge that this preliminary result serves as a predictor indicator, useful in a first potential screening. Further comprehensive tests, including *in vitro* and *in vivo* are needed to validate this observation.

The IC_{50} analysis reveals distinct cytotoxic profiles of the plant infusions across the three cell lines. In the non-tumour cell lines L929 (Figure 2A) and HEK293t (Figure 2B), most IC_{50} values exceeded 1000 µg/mL, indicating low cytotoxicity. Specifically, in HEK293t cells, the PB infusion showed an IC_{50} of 986 µg/mL (920–1051 µg/mL), while BT and BE presents a IC_{50} of 1680 and 1245 µg/mL, respectively, and CN exceeded 2000 µg/mL. In L929 cells, the BT and BE infusions showed IC_{50} values >2000 µg/mL, whereas PB and CN yielded IC_{50} of 1701 µg/mL (1490–1912 µg/mL) and 1486 µg/mL (1275 – 1697), respectively.

In contrast, the tumour-derived Caco-2 cells (Figure 2C) demonstrated significantly higher sensitivity. The PB infusion displayed an IC_{50} of 323 µg/mL (290–356 µg/mL), while BE and CN showed IC_{50} of 567 µg/mL (500–634 µg/mL) and 579 µg/mL (510–648 µg/mL), respectively. These findings suggest selective cytotoxicity of the plant infusions against cancer cells, particularly PB, which had the lowest IC_{50}.The disparity underscores the differential impact of infusions on cancer cells, suggesting a negative influence on cancerous cell lines. The higher IC_{50} values observed in non-tumour cells are likely due to the TPC present in the different plant infusions.

Upon analysing the composition of plant infusions, it was possible to observe that these plant Infusions are rich in antioxidant phenolic compounds including catechins, quercetins, kaempferol, phenolic acids, among others, as detailed in Table 2. These results show that plant infusions exhibit low toxicity at the tested concentrations and, to some extent, may have the ability to inhibit tumour cell growth. As described by Liu *et al.* [51] and Niero et al. [52], cinnamic acid can reduce cell proliferation of glioblastoma, melanoma, prostate and lung cancer. This can justify the results, as can be seen in Table 2, the extracts have a high concentration of cinnamic acid. But not only, as BE, extract with a higher concentration of cinnamic acid did not present the highest IC_{50}. Hesperidin has also been shown to be involved in the mechanism related to the inhibition of cancer cell proliferation by inducing apoptosis and cell arrest. But not only the authors mention the possibility of this compound to be involved in the inhibition tumor cell metastasis,

angiogenesis, and chemoresistance [53]. It is difficult to single out only one compound, for example is known that ellagic acid also has an anti-tumour effect in gastric cancer, liver cancer, pancreatic cancer, breast cancer, colorectal cancer lung cancer, among others. Literature reports mention its ability to inhibit tumour cell proliferation, and induce cancer cell apoptosis [54,55]. Similar results were described for naringin, which has been shown to inhibit the proliferation of tumor cells [56,57].

However, despite the promising results obtained, it is crucial to note that further research is imperative to delve deeper into the anticancer potential of these plant infusions. This is necessary due to the complexity of their composition and the need for a more comprehensive understanding of their action mechanisms and effects on cancer cells.

Figure 2.

3.4. Studies on cell death: apoptosis and cell cycle analysis

As mentioned previously, several compounds present in plant infusions can influence the cell cycle of cancer cells. Therefore, further studies were performed using the cancerous cell Caco-2. For that. flow cytometry analysis on cancer upon 48 h incubation was performed. Staining using Annexin-V/Propidium iodide (AV/PI) was used as it is a well-established biomarker of cell death [58]. PI is a well-known nuclear dye that binds to cells with reduced cell membrane permeability, specifically late apoptotic and necrotic cells. AV is an indicator of both early and late apoptosis as it attaches to the phosphatidylserine that moves to the outer layer of the cell membrane during apoptosis. AV is commonly employed in combination with PI to distinguish between early apoptosis (AV+/PI-), late apoptosis (AV+/PI+), and necrosis (AV-/PI+) based on variations in plasma membrane integrity and permeability [59].

The IC_{50} at 48 hours was determined to establish a reference concentration. As it can be seen in Figure 3A the treatment with the different plant infusions led to a higher level of apoptosis when compared to non-treated cells. In this sense, these findings demonstrated that all prepared plant infusions were able to induce Caco-2 cell death by activating apoptotic pathways.

Apoptosis, characterized as a programmed cell death process, is orchestrated through several signalling pathways initiated by multiple factors, including cellular stress, DNA damage, and

408 immune surveillance. These pathways converge to ensure a selective and controlled cell death,
409 making apoptosis the preferred outcome in cancer therapy [60,61]. The observed induction of
410 apoptosis by the plant infusions underscores their potential as promising candidates for further
411 investigation in the development of targeted and effective anticancer therapies, mainly as a co-
412 adjuvant in chemotherapy [62–64].
413 To investigate whether apoptosis contributed to cell growth inhibition, the impact of the plant
414 infusions on the cell-cycle progression of Caco-2 cells upon 48 h of treatment was investigated.
415 Therefore, flow cytometry was employed for cell-cycle analysis, evaluating the DNA content of
416 cells stained with PI. When compared to the control, a significant increase in the S phase was
417 observed for all studied plant infusions. In contrast, the G2/M phases exhibited results that
418 reassembled those of the control for all plant infusions. On the other hand, a decrease in the
419 G0/G1 phase was observed for all plant infusions when compared to the control (Figure 3B).
420 Although the effect of infusions on cell cycle regulation has not been widely reported, the
421 beneficial effects of tea as a chemopreventive agent for cancer have been recognized throughout
422 the past two decades. Recent suggestions propose that polyphenols have a strong ability to
423 trigger apoptotic cell death and cell cycle arrest specifically in tumour cells [50]. The results
424 presented in the current study align with previous findings on tea polyphenols treatment, which
425 demonstrated cell cycle arrest in the S phase for NOZ cells (metastatic gallbladder cancer) [65],
426 SGC-7901 cells (gastric cancer) [50] and on HeLa (cervix cancer) treated with *Primula vulgaris*
427 extract [66]. Moreover, it was shown that marjoram water extract led to the upregulation of cyclin-
428 dependent kinase inhibitor 1 (p21), leading to apoptosis and suppression of the cell cycle in the
429 breast cancer MCF-7 cell line. The authors correlate this result with the high content of phenolics
430 compounds [49].
431 In a comprehensive review paper, Maru *et al.* [19] described that chemoprevention is one of the
432 cancer prevention approaches wherein natural or synthetic agents are prescribed to delay or
433 disrupt multiple pathways and processes involved in cancer, *i.e*., initiation, promotion, and
434 progression. Amongst natural sources of chemopreventive compounds, dietary components are
435 being evaluated because of their wide human use, high tolerability, low toxicity and recorded
436 biological effects. This document compiles the chemopreventive efficacy and probable
437 chemoprevention mechanism of certain biomolecules present in foodstuffs (such as capsaicin,

curcumin, genistein, tea polyphenols, lycopene, resveratrol, tocopherols, etc.) in experimental systems and clinical trials. All the food phytochemicals discussed in this review have demonstrated chemopreventive efficacy against experimental tumours and/or biomarkers and associated processes in various organs. The observed activity of the biomolecules involves redox changes, modulation of enzymes and signalling kinases that result in effects on multiple genes and cell signalling pathways.

Figure 3.

3.5. ROS intracellular levels

Oxidative stress, known to damage biologically relevant molecules such as DNA, membrane lipids, or proteins, leading to cellular oxidative damage, can result from an imbalance between the formation of reactive oxygen species (ROS) and antioxidant defense mechanisms [67,68]. Moderate levels of ROS are essential for cellular proliferation, differentiation, and survival [69]. However, excessive ROS production influences several chronic diseases such as cardiovascular disorders, cancer, and diabetes [70] and premature aging [71]. Exploring alternative approaches to prevent their increase in the body has been as area of research [31].

To assess the ability of the plant infusions to prevent an excessive ROS formation upon contact with an oxidant agent TBHP, cells were previously placed in contact with the extracts and ROS production was evaluated. As can be seen in Figure 3C, all plant infusions were able to significantly reduce the formation of ROS species in both cell lines compared when compared to the positive control (cells incubated with TBHP). This was an expected result due to the high content of phenolics observed in each infusion, which corroborates the data described in Figure 1. As depicted in Figure 3C, the BE infusion demonstrates the highest ability to prevent ROS formation, which is correlated with its high phenolic content. As shown in Figure 1, BE has the highest concentration of phenolics among the infusions.

This is a very important result, according to Ziaidieh *et al.* [72] cancer cells with low levels of ROS may be more sensitive to treatment, while cells with high levels of ROS may be more resistant. As a hypothesis, the ability to significantly reduce ROS levels in cells may make them more sensitive to chemotherapy.

The cumulative data from multiple human epidemiological and clinical studies on tea consistently confirms its chemopreventive benefits, a phenomenon supported by tests conducted on cells and animals [47,73]. The findings of our study are consistent with previous research on polyphenols found in tea leaves, which have been extensively documented in scientific literature for their anti-tumor effects [74]. These findings reveal the beneficial antioxidant capabilities of plant infusions and emphasize their potential as useful resources in the search for preventive strategies against health hazards linked to oxidative stress.

3.6. Anti-inflammatory activity

Inflammation is the body's defence against biological (microorganisms, etc), chemical (heavy metals, smoking and other toxic substances), or physical agents (UV radiation, excessive exercise, etc). The overproduction of pro-inflammatory mediators by the immune system characterizes chronic inflammation [16]. Chronic inflammation promotes the development of cancer by activating inflammatory cells and causing an excessive generation of pre-inflammatory mediators like transcription factors such as Nuclear factor kappa-light-chain-enhancer of activated B cells (NF-kB) [75]. In addition, the activation of NF-kB enhances the production of pro-inflammatory cytokines (such as IL-1α, IL-1β, IL-6, IL-10, and TNF-α), chemokines (IL-8, macrophage inflammatory protein 1α, monocyte chemoattractant protein 3), adhesion molecules (intercellular adhesion molecules 1 (ICAMs), and vascular cell adhesion molecule 1 (VCAM-1)), cytosolic phospholipase, Inducible Nitric Oxide Synthase (iNOS) and Cyclooxygenase-2 (COX-2) [76,77].

Therefore, it is crucial to slow down the inflammatory process, mainly with daily diet, and natural products (*e.g.,* infusions, nutraceuticals, or functional foods) that rarely have adverse side effects [78]. Moreover, antioxidant compounds, such as vitamins and phenolics, also show promise as therapeutic agents because they minimize oxidative stress and regulate inflammatory pathways [79].

To understand whether the treatments did not show cytotoxic activity for the *in vitro* anti-inflammatory bioassay, the possible cytotoxicity of the infusions for all treatments on J774A.1 cells was determined followed by an MTT assay. No treatment had a significant increase or decrease in the number of viable cells compared to the control. It was only found that the LPS +

CN plant infusion treatment significantly ($p < 0.05$) stimulated cell proliferation compared to LPS in non-inflamed cells, but not in inflamed cells (Figure 4F and 5F).

it is well known that activated macrophages and neutrophils (*e.g.* with LPS) induce oxidative stress through the production of ROS and nitrogen species. Therefore, taking into account that phenolic compounds have antioxidant properties and observing the results obtained for the ROS tests on Caco-2 cells (section 3.5), it may be that the infusions act to attenuate the excessive production of free radicals and mitigate inflammatory reactions.

To assess the anti-inflammatory potential of plant infusions, the production of pro-inflammatory cytokines (TNF-α, IL-1β, IL-6) and two cytokines that can act as pro- or anti-inflammatory biomarkers (IL-10 and TGF-β1) were analyzed in macrophages. Pro- and anti-inflammatory cytokines promote and inhibit inflammation, respectively [80].

Our study reported the influence of plant infusions in the presence and absence of inflammation (normal and inflammation cases, respectively) to understand if plant infusions induce or prevent inflammation. In either case, the cells were incubated with the plant infusions in both the presence and absence of a potent activator of monocytes and macrophages that promote the production of inflammatory cytokines – LPS [81,82].

In non-inflamed macrophages, *i.e.,* without LPS overnight stimulus (results present in Figure 4), the TNF-α (Figure 4A) shows higher concentrations when compared to the control cells. In the presence and absence of LPS, all the infusions studied were unable to reduce the concentration of this biomarker. The increase in the TNF-α levels in the presence of LPS, may be due to the fact that the secretion of this cytokine is one of the first responses of the innate immune system to infection or tissue damage [81]. A previous study found similar results, but was unable to provide a precise explanation [66]. Similar results were achieved by Genc *et al.* [83] who reported that nitric oxide (NO) and TNF-α levels in macrophage cells (RAW264.7) decreased with treatment using *Plantago subulata* L. extract, which is rich in phenolic compounds, compared to cells incubated with LPS alone.

As we can see in Figure 4B, the presence of LPS increases the expression of IL-1β (around 80%) compared to the control cells. Infusions of plants without LPS do not promote the expression of this interleukin. When in the presence of LPS together with the infusions, there is a significant

reduction in the levels of this pro-inflammatory cytokine compared to LPS, for the 4 infusions tested.

Concerning IL-6 (Figure 4C), in the absence of LPS, the action of plant infusions is similar to the control. All infusions show similar concentrations for this cytokine, presenting values similar to LPS. Thus, this study demonstrated that formulated plant infusions can reduce the pro-inflammatory cytokine IL-1β.

Regarding anti-inflammatory cytokine IL-10 (Figure 4D), plant infusions demonstrate an increase in this biomarker compared to the control cells, observing significant values for CN infusions. In the presence of LPS, all plant infusions promote a significant increase in the expression of this anti-inflammatory cytokine. These results demonstrate the capacity of these formulated infusions to produce this cytokine, which is important for maintaining normal tissue homeostasis and protecting the body from an uncontrolled immune response. For TGF-β1 (Figure 4E), there were no significant differences between the all-tested groups.

Figure 4.

In the presence of inflammation (Figure 5), LPS promotes a significant increase in the expression of all pro-inflammatory cytokines (TNF-α, IL-1β, and IL-6) compared to the control cells. Plant infusions treatment permits the reduction of the expression of these cytokines compared to LPS, especially the IL-1β (Figure 5B). PB is the infusion with the highest capacity to reduce IL-1β levels expression. Contrary to the results obtained for the nom-inflamed macrophages, the infusions do not induce (or prevent) the production of TNF-α (Figure 5A), which is a positive aspect since it is a pro-inflammatory cytokine in inflammatory processes.

Relative to IL-6 concentrations (Figure 5C) do not present a significant difference for any of the infusions, compared to the control cells. Furthermore, LPS induces the production of this pro-inflammatory cytokine, which suggests the anti-inflammatory action of the infusions tested.

Similar to our results, in another anti-inflammatory study performed in macrophages (RAW264.7) by Hamsalakshmi *et al.* [82], the LPS treatment (1 μg/mL) led to a significant increase in the transcript levels of IL-6 and TNF-α when compared to the control cells. Pre-treatment of cells with the *Trichodesma indicum* (L.) plant extracts significantly reduced the IL-6 and TNF-α gene expression levels compared to LPS alone.

Several studies have demonstrated that phenolic compounds, particularly flavonoids, can inhibit regulatory enzymes or transcription factors important for controlling mediators involved in inflammation. Certain flavonoids can inhibit the production of pro-inflammatory cytokines by binding to cytokine receptors in mast cells, neutrophils and other immune cells [78].

For IL-10 (Figure 5D), only CN infusion shown significant increase in the production of this anti-inflammatory cytokine. This result suggests an improvement in immune regulation and an anti-inflammatory potential with an increase in IL-10 that occurred in both the inflamed and non-inflamed models. Regarding to TGF-β1 (Figure 5E), the cells treated with LPS showed an increase in this marker, with all the infusions tested showing lower values of this cytokine and concentrations similar to those of the control cells.

Several research groups have joined forces to understand the action of certain phenolic compounds on the inflammatory processes associated with certain pathologies. Neyestani *et al.* [17] studied the effects of cytokine secretion on human peripheral blood mononuclear cells treated with black tea extract and its pure phenolics (like gallic acid, epigallocatechin, caffeine and epigallocatechin gallate). Their results demonstrate that pure phenolics (20 mM) don't affect pro-inflammatory cytokine secretion, but the black tea infusion suppressed IL-1β, TNF-α and IL-6. These results suggest the beneficial synergetic effect of compounds against inflammatory responses. In this way, the consumption of infusions can be positive compared to isolated molecules.

In another study that used an *in vivo* model of diabetes in rats, cinnamic acid (a compound found in abundance in the infusions studied in our work) seems to attenuate the complications associated with the diabetic pathological state like cholesterol, triglycerides, insulin resistance, myocardial injury, etc. [40]. Moreover, inflammatory mediators such as TGF-β and beta-myosin heavy chain (β-MHC) in the hearts of diabetic rats, and pro-inflammatory cytokines (TNF-α, IL-6) and lipid peroxides in the serum of diabetic rats were also attenuated by the administration of cinnamic acid at doses of 5 and 10 mg/kg body weight [40]. Additionally, other works have shown that rutin increases the expression of antioxidant enzymes and can reduce pro-inflammatory cytokines and ROS [43,84].

Figure 5.

585 Overall, these results demonstrate that the studied infusions do not induce inflammation. In terms
586 of their therapeutic effect, the phenolic compounds present in the infusions have anti-
587 inflammatory capacity, both by inducing the production of anti-inflammatory cytokines and by
588 inhibiting pro-inflammatory cytokines. However, further studies are needed to confirm whether the
589 anti-inflammatory activity of compounds present in the formulated infusions is affected by human
590 metabolism.
591
592
593 **4. Conclusions**
594 People are drinking plant infusions for more than just taste. Their high nutrient content,
595 especially their polyphenols and antioxidants, makes them very important for health reasons. The
596 in-depth phytochemical analysis in this study showed that the plant infusions contained many
597 different compounds, such as phenolic acids, flavonoids, flavonols, and stilbenes.
598 To fully understand the complex nature of antioxidant activity, a series of tests were carefully
599 carried out using optical methods like Total Phenolic Content (TPC) and antioxidant activity using
600 different methods (ABTS, DPPH, and FRAP). The results uniformly attested to its richness in
601 phenolic compounds as well as their considerable antioxidant potential among all infusions, with
602 the BE infusion exhibiting the highest radical scavenging and ferric reduction efficacy.
603 In addition to being powerful antioxidants, the plant infusions showed low cytotoxicity in normal
604 cells and selective action in colon cancer cells (Caco-2). An important finding was a selective
605 effect on cancer cells when compared to normal cells.
606 The infusions have an anti-inflammatory capacity tested in macrophages, principally with a
607 representative decrease of pro-inflammatory cytokine IL-1β and an increase of anti-inflammatory
608 cytokine IL-10.
609 These findings not only accentuate the safety profile of plant infusions but also underscore their
610 potential applications in medical and nutraceutical domains, such as chemopreventive dietary
611 compounds, opening avenues for further exploration and development.

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Javier Quero: Writing – original draft, Investigation.

Augusto Costa-Barbosa: Investigation.

Catarina Teixeira-Guedes: Writing – original draft,

Paula Sampaio: Methodology, Investigation.

María Jesús Rodríguez-Yoldi: Writing – original draft, Investigation, Methodology.

Pedro Ferreira-Santos: Conceptualization, Investigation, Methodology, Project administration, Funding acquisition, Writing – review & editing, Writing – original draft.

Declaration of 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.

Figure captions

Figure 1. Total phenolic content (TPC) and antioxidant activity (DPPH, ABTS and FRAP assay) of plant infusions.

Figure 2. Cell viability of HEK293t (**A**), L929 (**B**) and Caco-2 (**C**) cells after incubation with plant infusions (0, 62, 125, 250, 500, 1000 and 2000 µg/mL) for 48 h.

Figure 3. Measurements of apoptosis (**A**), cell cycle arrest (**B**), and reactive oxygen species (ROS) (**C**) of Caco-2 cells after incubation with plant infusions (IC_{50}) for 48 h. * corresponds to a statistical difference between control cells, and # corresponds to a statistical difference between TBHP pretreated cells.

Figure 4. Measurements of cytokines in non-inflamed macrophages after incubation with or without LPS and plant infusions (2500 µg/mL) for 24h. (**A**) tumor necrosis factor-α (TNF-α), (**B**) interleukin-1β (IL-1β), (**C**) interleukin-6 (IL-6), (**D**) Interleukin-10 (IL-10), and (**E**) transforming Growth Factor-Beta 1 (TGF-β1). * corresponds to a statistical difference between control cells, # corresponds to a statistical difference between LPS, and ϕ corresponds to a statistical difference between LPS+plant infusion treated cells and cells treated with the same plant infusion without LPS.

Figure 5. Measurements of cytokines in inflamed macrophages after incubation with or without LPS and plant infusions (2500 µg/mL) for 24h. (**A**) tumor necrosis factor-α (TNF-α), (**B**) interleukin-1β (IL-1β), (**C**) interleukin-6 (IL-6), (**D**) Interleukin-10 (IL-10), and (**E**) transforming Growth Factor-Beta 1 (TGF-β1). * corresponds to a statistical difference between control cells, # corresponds to a statistical difference between LPS, and ϕ corresponds to a statistical difference between LPS+plant infusion treated cells and cells treated with the same plant infusion without LPS.

Figure 1.

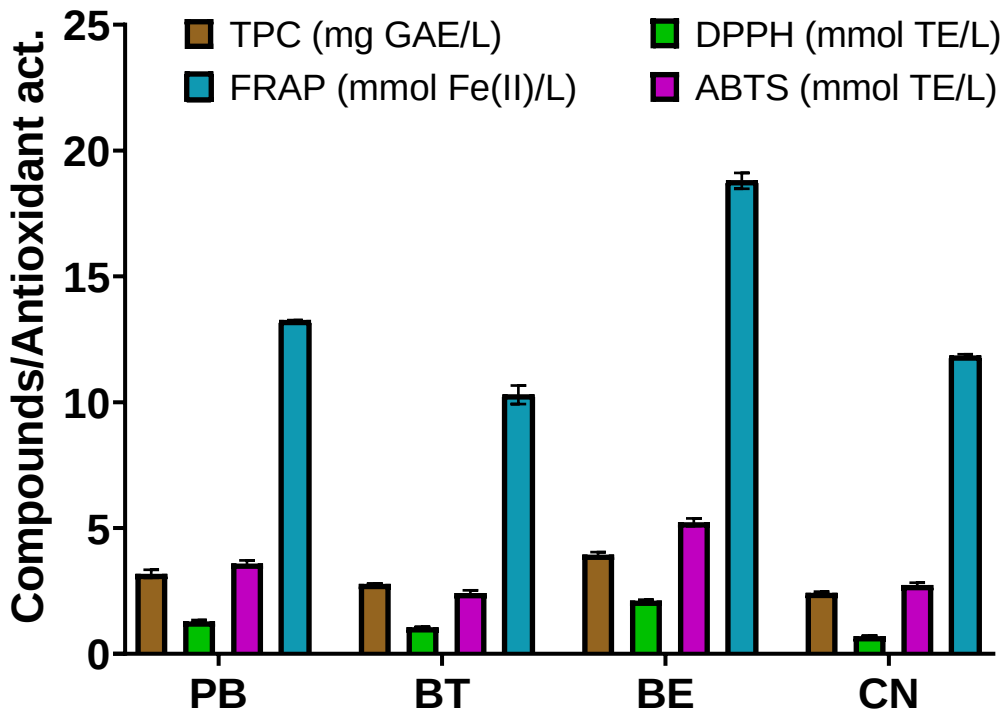
Compounds/Antioxidant act.
25
20
15
10
5
0
TPC (mg GAE/L)
DPPH (mmol TE/L)
FRAP (mmol Fe(II)/L)
ABTS (mmol TE/L)
PB
BT
BE
CN

Figure 2.

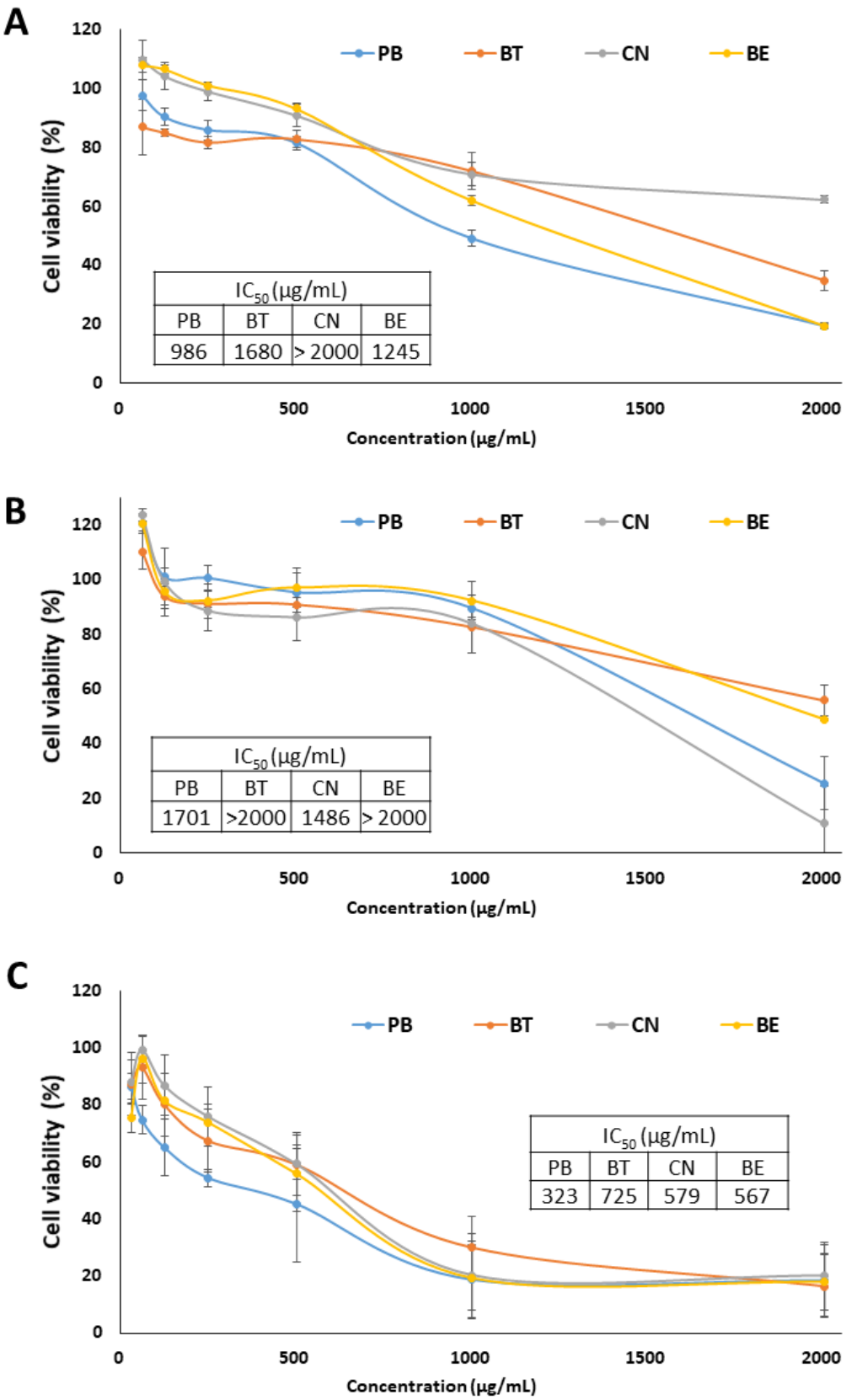
A
PB
BT
CN
BE
Cell viability (%)
Concentration (µg/mL)
IC50 (µg/mL)
PB BT CN BE
986 1680 > 2000 1245
B
PB
BT
CN
BE
Cell viability (%)
Concentration (µg/mL)
IC50 (µg/mL)
PB BT CN BE
1701 >2000 1486 > 2000
C
PB
BT
CN
BE
Cell viability (%)
Concentration (µg/mL)
IC50 (µg/mL)
PB BT CN BE
323 725 579 567

Figure 3.

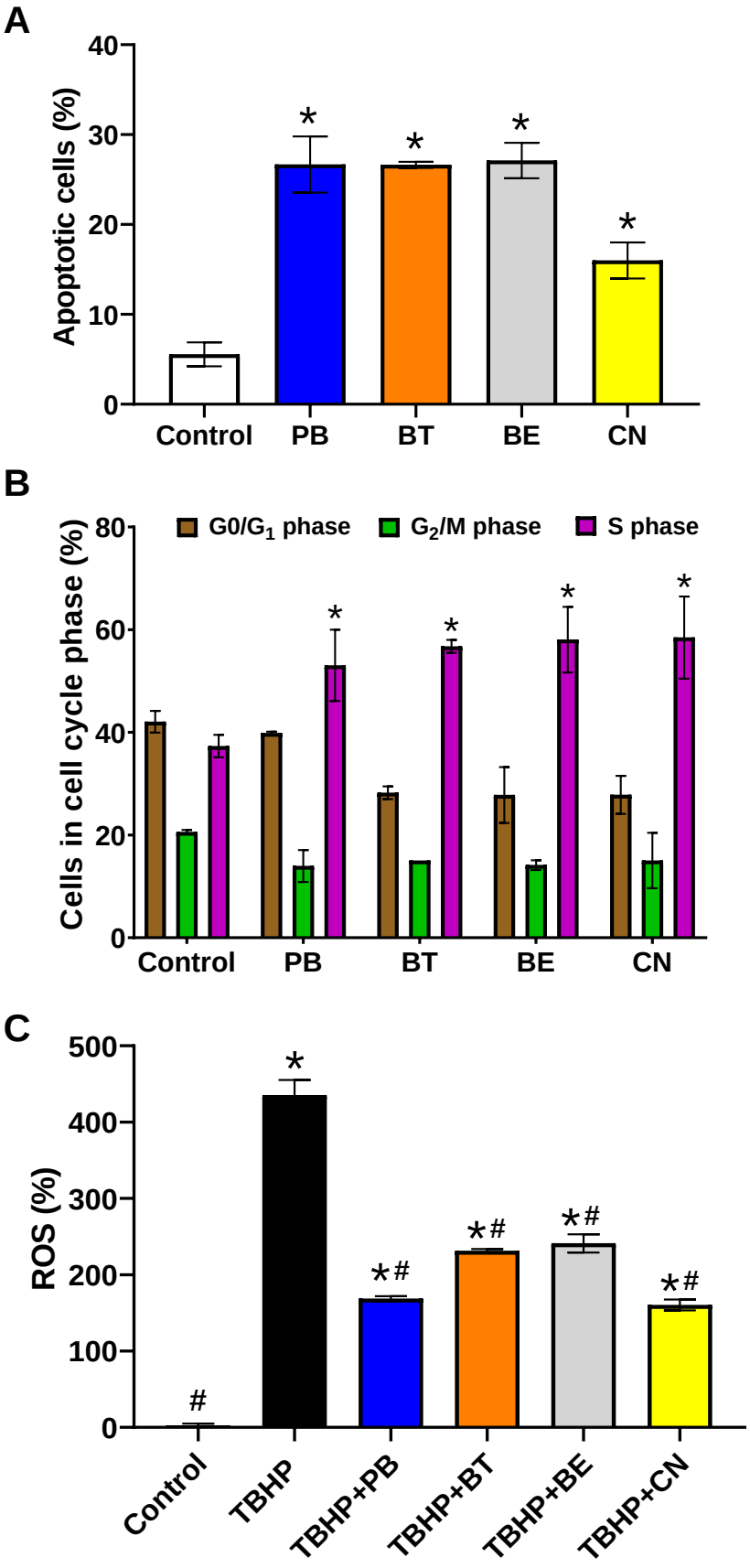
A
Apoptotic cells (%)
0
10
20
30
40
*
*
*
*
Control
PB
BT
BE
CN
B
Cells in cell cycle phase (%)
0
20
40
60
80
G0/G1 phase
G2/M phase
S phase
*
*
*
*
Control
PB
BT
BE
CN
C
ROS (%)
0
100
200
300
400
500
#
*
*#
*#
*#
Control
TBHP
TBHP+PB
TBHP+BT
TBHP+BE
TBHP+CN

Figure 4.

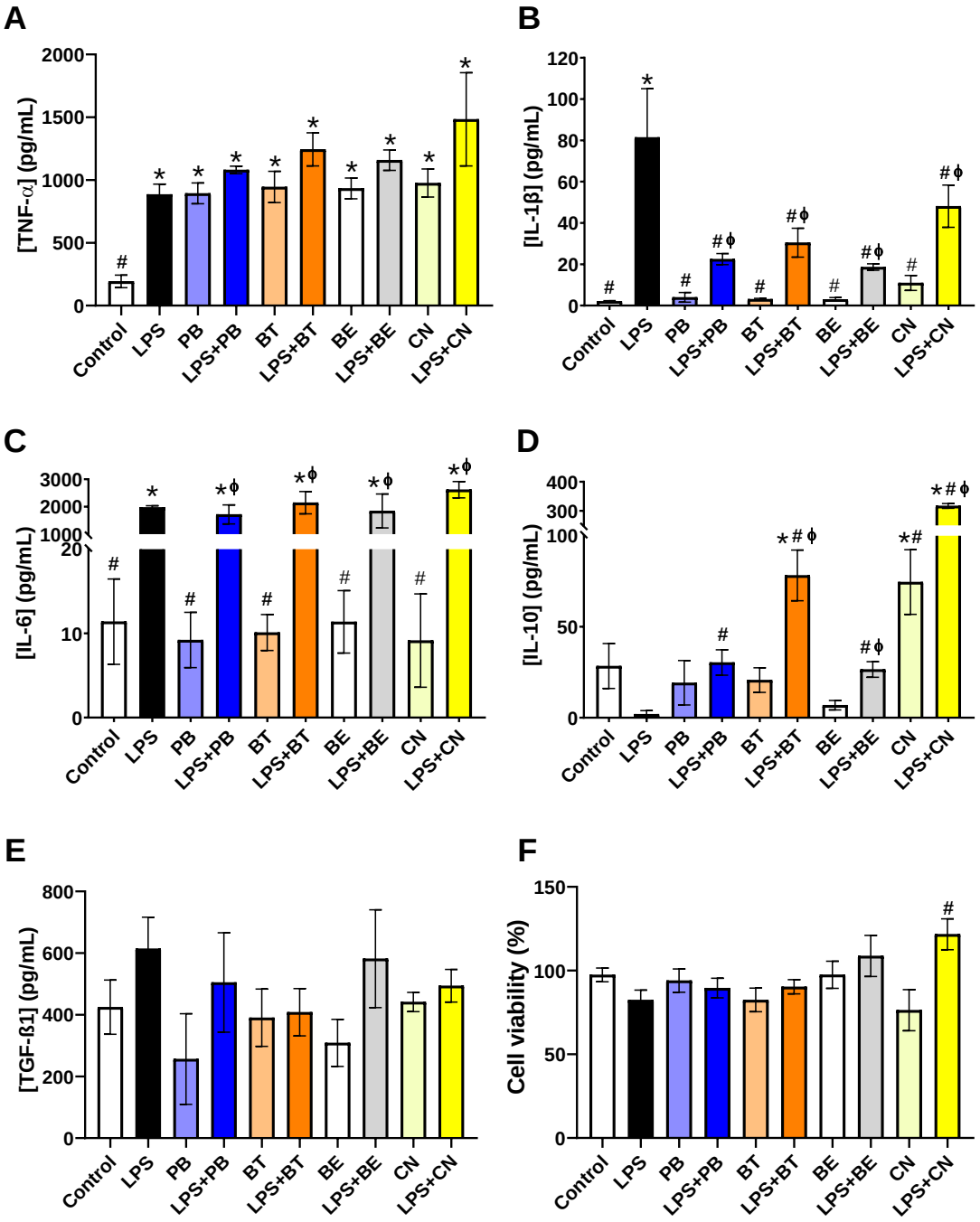
A
[TNF-α] (pg/mL)
2000
1500
1000
500
0
B
[IL-1β] (pg/mL)
120
100
80
60
40
20
0
C
[IL-6] (pg/mL)
3000
2000
1000
20
10
0
D
[IL-10] (pg/mL)
400
300
100
50
0
E
[TGF-ß1] (pg/mL)
800
600
400
200
0
F
Cell viability (%)
150
100
50
0
Control
LPS
PB
LPS+PB
BT
LPS+BT
BE
LPS+BE
CN
LPS+CN

Figure 5.

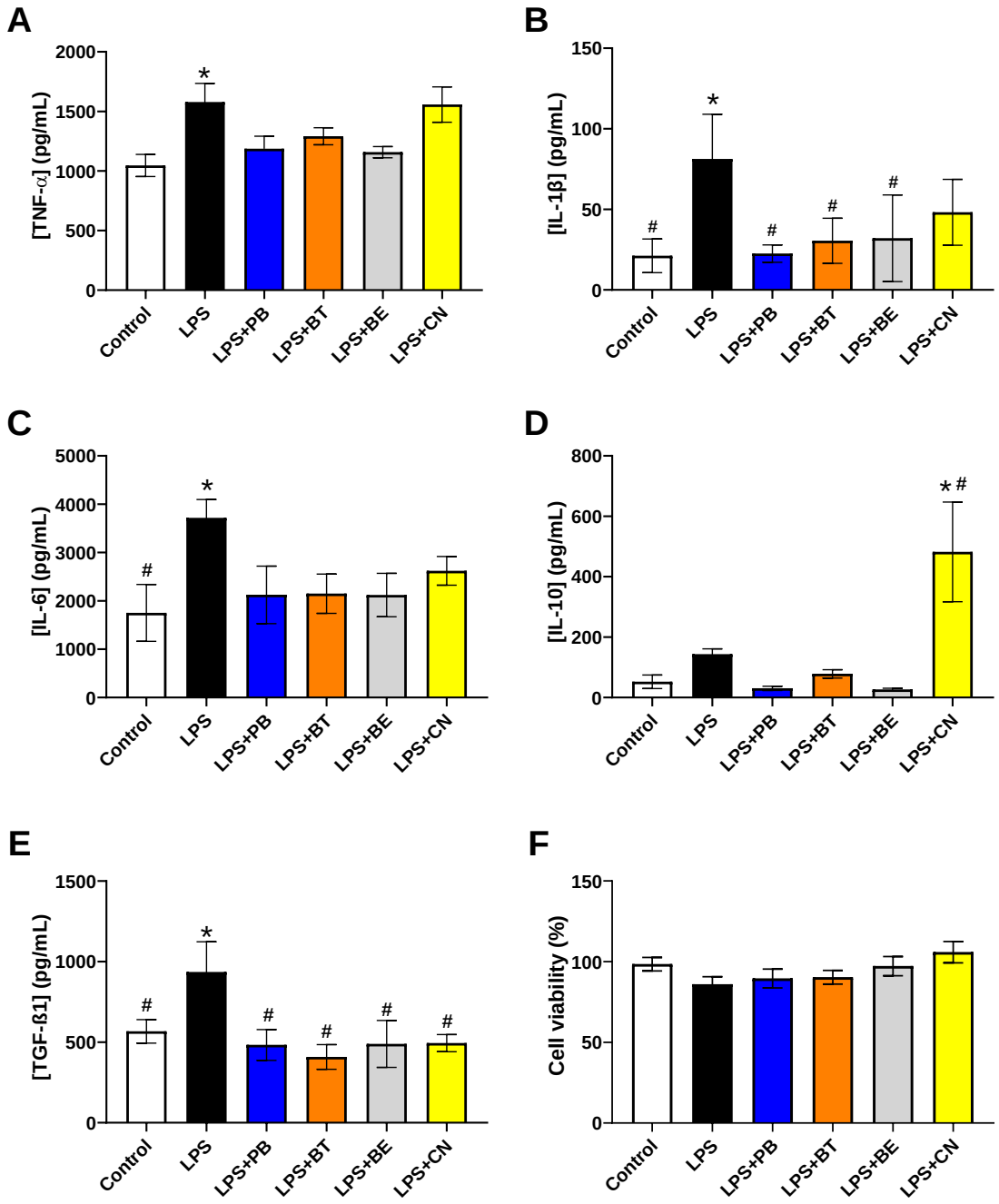
A
[TNF-α] (pg/mL)
2000
1500
1000
500
0
*
Control
LPS
LPS+PB
LPS+BT
LPS+BE
LPS+CN
B
[IL-1β] (pg/mL)
150
100
50
0
*
#
C
[IL-6] (pg/mL)
5000
4000
3000
2000
1000
0
*
#
D
[IL-10] (pg/mL)
800
600
400
200
0
*#
E
[TGF-ß1] (pg/mL)
1500
1000
500
0
*
#
F
Cell viability (%)
150
100
50
0

Table 1

Table 1. Plant composition of formulated infusions.

Infusion name	**Purifying Body**	**Boost Training**	**Calm Night I Spirit**	**Boost Energy**
Plant composition	*Cichorium intybus* L. (17%)	*Illicium verum* Hook. (17%)	*Foeniculum vulgare* Mill. (17%)	*Rosmarinus officinalis* L. (17%)
	Betula alba L. (17%)	*Centella asiatica* L. (17%)	*Lavandula angustifolia* Mill. (17%)	*Calendula officinalis* L. (17%)
	Peumus boldus Molina (17%)	*Camellia sinensis* (L.) Kuntze (17%)	*Lippia triphylla* Kuntze (17%)	*Equisetum arvense* L. (17%)
	Cochlospermum angolensis (17%)	*Phaseolus vulgaris* L. (17%)	*Jasminum grandiflorum* L. (17%)	*Camellia sinensis* (17%)
	Taraxacum officinale Web. (16%)	*Paullinia cupana* Kunth (16%)	*Passiflora incarnata* L. (16%)	*Ginkgo biloba* L. (16%)
	Cassia angustifolia Vahl. (16%)	*Calluna vulgaris* L. (16%)	*Verbena officinalis* L. (16%)	*Eleutherococcus senticosus* Maxim (16%)

Table 2

Table 2. Phenolic compounds identification and quantification of plant infusions.

Name	Chemical formula	Classification	Samples			
			PB	BT	BE	CN
Vanillic acid	$C_8H_8O_4$	HBA	n.d.	n.d.	0.34 ± 0.03	n.d.
Cinnamic acid	$C_9H_8O_2$	HCA	1203 ± 8	176.1 ± 26	1900 ± 100	1262 ± 16
Caffeic acid	$C_9H_8O_4$	HCA	2.12 ± 0.1	6.88 ± 0.3	9.87 ± 0.6	6.06 ± 0.4
Catechin	$C_{15}H_{14}O_6$	Flavanol	n.d.	3.68 ± 0.1	13.45 ± 1.5	175.42 ± 9.4
Epicatechin	$C_{15}H_{14}O_6$	Flavanol	9.04 ± 0.3	n.d.	7.48 ± 0.1	n.d.
***o*-coumaric acid**	$C_9H_8O_3$	HCA	20.25 ± 3.1	11.20 ± 1.4	15.24 ± 0.2	7.55 ± 0.9
Rosmarinic acid	$C_{18}H_{16}O_8$	HCA	28.56 ± 8.4	8.66 ± 1.0	75.07 ± 6.4	16.43 ± 0.3
Ellagic acid	$C_{14}H_6O_8$	HBA	78.59 ± 3.6	8.82 ± 0.5	61.83 ± 3.5	39.57 ± 2.8
Naringin	$C_{27}H_{32}O_{14}$	Flavanone	74.83 ± 6.7	51.10 ± 2.0	24.50 ± 3.5	27.69 ± 2.1
Hesperidin	$C_{28}H_{34}O_{15}$	Flavanone	70.72 ± 7.2	11.99 ± 1.1	139.52 ± 4.6	17.96 ± 1.3
Gallic acid	$C_7H_6O_5$	HBA	n.d.	27.97 ± 1.2	29.69 ± 1.2	22.54 ± 1.1
Resveratrol	$C_{14}H_{12}O_3$	Stilbene	4.20 ± 0.5	2.44 ± 0.9	4.77 ± 0.6	6.84 ± 1.0
Ferulic acid	$C_{10}H_{10}O_4$	HCA	23.10 ± 0.5	2.15 ± 0.3	10.97 ± 1.1	5.14 ± 1.1
Rutin	$C_{27}H_{30}O_{16}$	Flavonol	62.17 ± 2.5	34.43 ± 1.8	83.80 ± 4.7	19.94 ± 1.8
Quercetin	$C_{15}H_{10}O_7$	Flavonol	3.55 ± 0.3	0.75 ± 0.1	3.71 ± 0.2	1.42 ± 0.2
3,4-DHB acid	$C_7H_6O_4$	HBA	6.46 ± 0.1	9.32 ± 0.6	18.12 ± 0.4	9.41 ± 0.6
Taxifolin	$C_{15}H_{12}O_7$	Flavonol	81.24 ± 0.4	7.50 ± 0.4	31.85 ± 2.0	11.17 ± 2.0
2,5-DHB acid	$C_7H_6O_4$	HBA	11.40 ± 0.0	12.71 ± 0.2	11.84 ± 0.7	n.d.
Kaempferol	$C_{15}H_{10}O_6$	Flavonol	3.59 ± 0.4	n.d.	6.03 ± 0.3	0.39 ± 0.1

Values of phenolic compounds are expressed as concentration (mg/L) mean ± SD of 3 experiments. n.d.: not detected; DHB: Dihydroxybenzoic; HBA: Hydroxybenzoic acid; HCA: Hydroxycinnamic acid.