International Food Research Journal 28(6): 1187 - 1195 (December 2021)
Journal homepage: *http://www.ifrj.upm.edu.my*

Antioxidant and pro-apoptosis activities of coffee husk (*Coffea arabica*) anthocyanins

[1]Fu, X. P., [1]*Shen, X. J., [1]Yin, X., [1]Zhang, Y. H., [1]Wang, X. F., [2]Han, Z. H., [1]Lin, Q. and [1]Fan, J. P.

[1]College of Food Science and Technology, Yunnan Agricultural University, Kunming 650201, China
[2]College of Food Science and Engineering, Qilu University of Technology, Jinan 250353, China

Article history

Received: 2 November 2020
Received in revised form: 18 March 2021
Accepted: 10 May 2021

Keywords

coffee husk anthocyanins, Coffea arabica, antioxidant, pro-apoptosis properties, flow cytometry

Abstract

The commercial use of coffee (*Coffea arabica*) husks, one of the major solid residues obtained during the dry processing of coffee, has previously been considered unprofitable. However, coffee husks are an excellent source of stable anthocyanins that have antioxidative, anti-inflammatory, and cardioprotective properties. The present work identified two anthocyanins, cyanidin-3-glucoside and cyanidin-3-rutinoside, from coffee husks by high-performance liquid chromatography and liquid chromatography-mass spectrometry. The antioxidant capabilities were tested by quenching free radical scavenge assay, reducing power, and ORAC assay at 50, 100, 150, 200 mg/L of coffee husk anthocyanins (CHAs). Moreover, the effects of different concentrations of CHAs on superoxide dismutase (SOD) and lactate acid dehydrogenase (LDH), and the concentration of malondialdehyde (MDA) in human umbilical vein endothelial cells exposed to hydrogen peroxide were also evaluated. Human colon cancer (Caco-2) cell apoptosis induced by CHAs was examined by flow cytometry. Based on the results, CHAs showed strong dose-dependent antioxidant activities, and could increase SOD activity, and suppress indicators for oxidative injuries such as MDA and LDH. Furthermore, the proportion of apoptotic Caco-2 cells ranged from 4.12 to 41.3% in response to treatment with different concentrations of CHAs. These results suggest that CHAs exhibit antioxidant and pro-apoptosis activities.

Introduction

Coffee, made from the roasted seeds of *Coffea arabica*, a widely cultured crop, is a very popular hot beverage due to its attractive aroma and unique flavour (Wei *et al.,* 2012; Cagliani *et al.,* 2013). The popularity of coffee is also related to coffee's image as a functional food (De Melo Pereira *et al.,* 2020). Coffee production generates large amount of coffee by-products such as silverskin, parchment, pulp, husk, and skin; 30 - 50% of the total weight of the produced coffee predominantly ended up as waste in the past (Oliveira and Franca, 2015). Researchers have then begun to study the potential uses of coffee by-products to increase their utilisation. Studies showed that coffee by-products contain a number of biologically active compounds including caffeine, tannins, chlorogenic acid (Brendan and Tien, 2018), carotenoids (Moreira *et al.,* 2018), anthocyanins (Oliveira and Franca, 2015), and melanoidins (Tores de la Cruz *et al.,* 2019), all of which are beneficial for human health (Duangjai *et al.,* 2016; Amaia *et al.,* 2019). Recent research indicated that coffee by-products could be used as novel foods (Klingel *et al.,* 2020).

Coffee husks (CHs) are one of the major coffee by-products from the de-hulling process of the coffee cherries during drying. The CHs have been considered as an excellent novel source of stable anthocyanins (Prata and Oliveira, 2007) which are natural plant pigments of the flavonoid family. Flavonoids have been demonstrated to have the following activities: antioxidative, free radical scavenging, coronary heart disease preventive, hepatoprotective, anti-inflammatory, and anticancer (Shi *et al.,* 2019; Wenzel *et al.,* 2000). A number of flavonoids exhibit potential antiviral activities (Zeng *et al.,* 2007). Today, anthocyanins gained increasing interest as potent bioactive agents because of their oxidative (Gabriela *et al.,* 2018), anti-inflammatory (Lee *et al.,* 2018), and cardioprotective properties (Krga and Milenkovic, 2019). However, to date, only few studies have reported the antioxidant and pro-apoptosis potentials of coffee husk anthocyanins (CHAs) in different cell lines. Liquid chromatography-mass spectrometry (LC-MS), a powerful tool for chemical analysis, has been utilised for the quality evaluation of natural products based on the efficient

*Corresponding author.
Email: 690361382@qq.com (Shen, X. J.); 1993033@ynau.edu.cn (Fan, J. P.)
DOI: https://doi.org/10.47836/ifrj.28.6.10

chromatographic separation and accurate identification of individual compounds (Zhu *et al.,* 2013). With the availability of this method for qualitative and quantitative analyses, LC-MS has become one of the most frequently applied techniques for evaluating chemical profiles of botanical products (Yi *et al.,* 2013; Chen *et al.,* 2017).

To estimate the efficacy of CHAs, and to support further utilisation of coffee by-products, the CHAs were determined by HPLC and LC-MS. The main antioxidant effects of CHAs were evaluated *in vitro* by quenching their hydroxyl radical free scavenging activity, reducing power, and oxygen radical absorbance capacity (ORAC). Moreover, the effect of CHAs on the activities of superoxide dismutase (SOD) and lactate acid dehydrogenase (LDH), as well as the concentration of malondialdehyde (MDA) in human umbilical vein endothelial (HUVEC) cells exposed to hydrogen peroxide (H_2O_2) were also determined. In addition, following the treatment with CHAs, human colon cancer (Caco-2) cell apoptosis was examined by flow cytometry.

Materials and methods

Materials, chemicals, and reagents

Fresh coffee cherries were collected at Beihuigui coffee company in Puer and Baoshan cities, Yunnan, China, in September 2014. The coffee cherries were peeled, and the peel was homogenised to obtain the coffee husks (CHs).

The HUVEC and Caco-2 cell lines were purchased from ALLCELLS Biotechnology Company (Shanghai, China) and Kunming Institute of Zoology Cell Line Bank (Yunnan, China), respectively. Dulbecco's Modified Eagle's Medium (DMEM), foetal bovine serum, penicillin, and streptomycin were purchased from Invitrogen (Eugene, USA). Assay kits for SOD, LDH, and MDA were purchased from Jiancheng Bioengineering Institute (Jiangsu, China). An annexin V-FITC apoptosis detection kit was purchased from Beyotime Biotechnology Company (Shanghai, China).

High-performance liquid chromatography (HPLC)-grade methanol was purchased from Merck Inc. (Darmstadt, Germany). Gallic acid (purity > 98%) was purchased from Solarbio, Inc. (Beijing, China). Fluorescein was purchased from Sigma-Aldrich, Inc. (St Louis, USA). Na_2CO_3, $FeSO_4$, H_2O_2, trichloroacetic acid (TCA), 2,2′-azobis-2-methyl-propanimidamide (AAPH), and 2-tribarbituric acid (TBA) were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China), and were all of analytical grade.

Extraction of coffee husk anthocyanins

Coffee husks were immersed in 50% HCl-MeOH for 24 h, and this was repeated twice. Then, the extract solution was filtered, condensed by a rotary evaporator (RE-2000A, Shanghai, China) at 50°C, and stored in a refrigerator at -20°C until further analysis.

Determination of total phenolic contents of the extract

Briefly, 50 µL of extraction solution was mixed with 250 µL of Folin-Ciocalteu reagent and 2.5 mL of distilled water for 3 min. Then, 750 µL of 20% Na_2CO_3 solution was added, and the mixture was shaken for 60 min at room temperature. The absorbance was measured at 765 nm, using gallic acid as standard. The concentration of total phenolic contents in the sample was expressed as mg of gallic acid equivalents (GAE)/g of the extract.

Purification of coffee husk anthocyanins

The extract was subjected to MCI gel and CHP 20P gel column chromatography (CC), eluted with a CH_3OH-H_2O (5% acetic acid) gradient system to obtain fractions. Then, these fractions were repeatedly separated by Sephadex LH-20 CC and ODS-gel CC with CH_3OH-H_2O (5% acetic acid) gradient system to obtain CHAs. At the same time, the apoptosis-inducing capacity of the CHAs in Caco-2 cells was evaluated.

HPLC and LC-MS analysis of coffee husk anthocyanins

The CHAs were separated by an Agilent 1100 HPLC system, which was equipped with a UV detector (330 nm) and XBridge C18 column (250 × 4.6 mm, 5 µm particle size). The mobile phase was CH_3OH-H_2O from 10 to 90% at a flow rate of 1 mL/min. The column temperature was 30°C, injection volume was 20 µL, and separation time was 30 min. The CHAs were determined by HPLC coupled with a MS system (HPLC-MS) gel chromatograph using Thermo Scientific Quantum (San Jose, USA). Mass spectrometry was performed with a photodiode array detector and an electrospray ionisation (ESI) interface, operated in positive mode. The source parameters were as follows: capillary voltage of 1500 V, drying gas N_2 at a flow rate of 530 L/h, drying gas temperature of 105°C, and mass range of *m/z* 50 - 1500.

Hydroxyl radical scavenging capacity of coffee husk anthocyanins

The hydroxyl radical scavenging capacity of CHAs was determined according to Je *et al.* (2009). Hydroxyl radicals were generated by a Fenton reaction in the presence of $FeSO_4$. A reaction mixture, comprising 10 mM 2-deoxyribose, 10 mM $FeSO_4$, 10 mM EDTA, and 10 mM H_2O_2 in 0.1 M phosphate buffer (pH 7.4) was added to different concentrations of CHAs, and the mixtures were incubated for 4 h at 37°C. Then, the reaction was stopped by adding 0.5 mL of 2.8% trichloroacetic acid (TCA) and 1.0% of 2-tribarbituric acid (TBA). The sample solution was cooled in water after keeping it in a boiling water bath for 10 min. The absorbance was measured at 532 nm.

Reducing power ability of coffee husk anthocyanins

The reducing power ability of CHAs was determined according to Pavithra and Sasikumar (2015). Briefly, 1.0 mL of diluted CHAs solution was mixed with 0.5 mL of 0.2 M phosphate buffer (pH 6.6) and 2.5 mL of 1% potassium ferricyanide ($K_3Fe(CN)_6$), and the mixture was incubated in a water bath at 50°C for 30 min. Then, 0.5 mL of 10% trichloroacetic acid was added and centrifuged (13,000 *g*, 10 min). Next, 2.5 mL of the upper layer, 2.5 mL of distilled water, and 0.5 mL of 0.1% of ferric chloride were mixed. The absorbance was measured at 700 nm.

Oxygen radical antioxidant capacity of coffee husk anthocyanins

Oxygen radical antioxidant capacity (ORAC) assay of CHAs was performed according to Anthony and Saleh (2013) with minor modifications. First, 100 µL of CHAs were diluted by phosphate buffer (75 mM, pH 7.4) and later, 50 µL of 50 nM fluorescein was added and the mixture was placed into black 96-well microplates. Then, the reaction mixture was incubated at 37°C for 15 min. Finally, 20 mM of 2,2′-azobis-(2-methyl-propionamidine) hydrochloride (AAPH) solution was added to a final volume of 200 µL. The fluorescence signal was measured using a fluorescence spectrophotometer (BioTek synergy H4, Winooski, USA) at 10 min intervals for 120 min using excitation wavelength of 485 nm and emission wavelength of 520 nm. Trolox was used as standard for the construction of the calibration curve.

Cell culture

HUVEC cells were cultured in DMEM with 10% foetal bovine serum (FBS), 10,000 units/mL penicillin, and 50 µg/mL streptomycin at 37°C under a humidified atmosphere with 5% CO_2 supplementation. The Caco-2 cells were also cultured in DMEM with 10% FBS, 2 mM L-glutamine, 100 U/mL penicillin, and 0.1 mg/mL streptomycin at 37°C under a humidified atmosphere with 5% CO_2 supplementation.

Determination of superoxide dismutase and lactate acid dehydrogenase activities, and malondialdehyde concentrations

The activities of SOD and LDH, as well as the MDA concentrations were determined according to Fan *et al.* (2013). Briefly, 2×10^5 HUVEC cells were seeded in T-25 flasks, and cultured for 24 h at 37°C under a humidified atmosphere with 5% CO_2. Then, HUVEC cells were treated with different concentrations of CHAs (100, 200, and 300 mg/L in methanol) and cultured for 24 h. Next, 400 µmol/L of H_2O_2 was added, and the mixtures were cultured again for 24 h. At the end of the incubation period, the supernatants and cell lysates were collected. The activities of SOD and LDH, as well as the MDA concentration were detected using assay kits in accordance with the manufacturer's instructions. The absorbance (A) was read at 440 nm for LDH activity, 532 nm for MDA concentration, and 550 nm for SOD activity.

Apoptotic rate assay of Caco-2 cells

The proportion of apoptotic cells in CHAs-induced Caco-2 cells was detected using an annexin V-FITC apoptosis detection kit in accordance with the manufacturer's instructions. The Caco-2 cells were seeded in T-25 flasks, and treated with different concentrations of CHAs for 12 h. Then, cells were digested with trypsin for 2 min, harvested, and the cells were centrifuged (15,000 *g*, 15 min). The pellets were removed and washed twice with PBS, and re-suspended in 1× binding buffer. Finally, 5 µL of annexin V-FITC conjugate and 10 µ L of propidium iodide were added, and the cells were kept in the dark at room temperature for 10 min. The annexin V-FITC/PI-stained cells were assessed by flow cytometry.

Statistical analysis

All experiments were performed in triplicate, and SPSS 18.0 was used to analyse the data. The results were expressed as mean ± standard deviation (SD). One-way analysis of variance (ANOVA) was used to determine the differences among experimental groups, where $p < 0.05$ was considered as significant difference.

Results and discussion

Total phenolic content of coffee husks

The CHAs were extracted using 50% HCl-MeOH for 24 h, which was repeated twice. Results showed that CHs were rich in anthocyanins, which have received increasing interest as functional compounds in the food manufacturing industry. The phenolic content of CHs was 14.99 ± 0.029 (GAE)/g. The CHA content has been reported to be related to the extraction conditions such as pre-soaking, liquid ammonia pre-treatment, sonication, liquid-solid ratio, extraction temperature, extraction time, sample immersion time, and extraction times (Zhao *et al.,* 2020). The extraction conditions should be optimised by future studies to further increase the yield of CHAs.

HPLC and LC-MS characterisations of coffee husk anthocyanins

The HPLC-MS was used to analyse CHAs. Two peaks were detected at 520 nm in the HPLC chromatogram of CHAs (Figure 1). The chemical structures of these two anthocyanins were identified using LC-MS, and compared with the data published by Murthy and Madhava (2012). Cyanidin-3-glucoside and cyanidin-3-rutinoside were identified as the main CHAs, which agree with previous findings (Prata and Oliveira, 2007; Murthy and Madhava, 2012; Oliveira and Franca, 2015).

Hydroxyl radical scavenging capacity of coffee husk anthocyanins

Fruits and vegetables are rich in anthocyanins, and their free radicals scavenging and reducing power abilities have been confirmed (Szymanowska *et al.,* 2018; Zhu *et al.,* 2019). The antioxidant capabilities of CHAs were assessed by their scavenging ability of free radicals and reducing power ability. Moreover, the dose response of the quenching of hydroxyl free radical scavenging efficacy of CHAs was also evaluated. As shown in Figure 2, the scavenging rates of hydroxyl radicals by CHAs were 66.27 ± 8.48, 72.29 ± 13.40, 78.31 ± 0.96, and 84.58 ± 0.96% at 50, 100, 150, and 200 mg/L, respectively, when compared with ascorbic acid. These rates increased with increasing CHA concentrations. At 200 mg/L, CHAs showed the highest scavenging capability when compared with the other concentrations ($p < 0.05$).

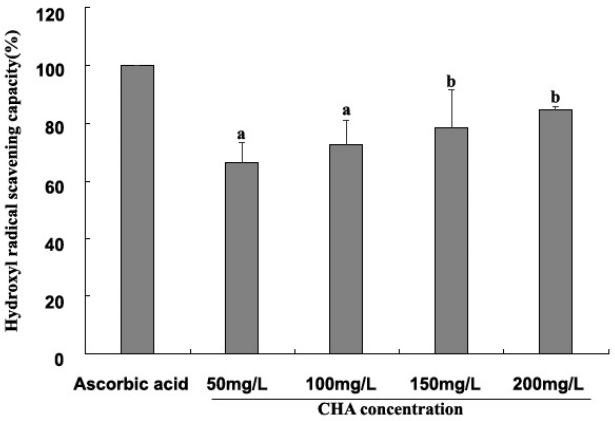

Figure 2. Hydroxyl radical scavenging activity of coffee husk anthocyanins (CHAs). Values are mean of triplicate ($n = 3$) with error bars indicating SD. Means with different lowercases indicate significant differences ($p < 0.05$).

Reducing power ability of coffee husk anthocyanins

The reducing power of bioactive components is often used as an indicator of phenolic

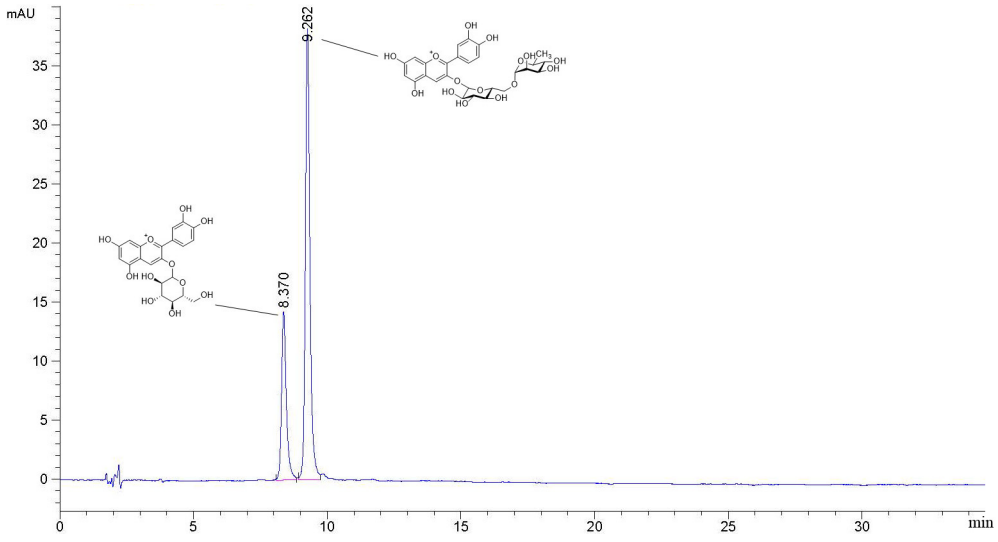

Figure 1. High-performance liquid chromatography (HPLC) chromatogram of coffee husk anthocyanins (CHAs).

antioxidant action (Orhan *et al.,* 2009). In the present work, the reducing power of CHAs, as detected by the Fe^{3+} reduction method, and a higher optical density (OD) value were used as indicators of a larger reducing power. As shown in Figure 3, the reducing powers of CHAs were 0.058 ± 0.002, 0.079 ± 0.004, 0.097 ± 0.002, and 0.119 ± 0.001 at 50, 100, 150, and 200 mg/L, respectively ($p < 0.05$). This showed that OD increased with increasing concentrations from 50 to 200 mg/L. The reducing power of CHAs was similar to the antioxidant activity which increased with the concentration of CHAs. Furthermore, the reducing power of CHAs was much lower than that of ascorbic acid, which ranged from 4.52 to 9.28%.

Oxygen radical absorbance capacity of coffee husk anthocyanins

The ORAC assay is based on the antioxidant inhibition of the peroxyl radical-induced oxidation of a sample, where the peroxyl radicals are generated by the thermal decomposition of azo-compounds, such as AAPH (Mellado-Ortega *et al.,* 2017). In the present work, the antioxidant capability of CHAs was also evaluated by ORAC assay. As shown in Figure 4, the oxygen radical absorbance capacity of CHAs also followed a dose-dependent relationship, and was strongest at 200 mg/L when compared with the other concentrations. The ORAC value of CHAs was 335.6 ± 0.06 μmoL TE/g, which matched the values reported by other studies on antioxidant activities of anthocyanins from different sources (Prior and Wu, 2006; Galvano *et al.,* 2007).

Based on these results, the antioxidant activity of CHs may be related to their anthocyanin contents, which showed high activities for 1,1-diphenyl-2-picrylhydrazyl (DPPH) scavenging, superoxide anion radical scavenging, hydrogen peroxide scavenging, reducing power, and metal chelating on ferrous ions (Steed and Truong, 2008).

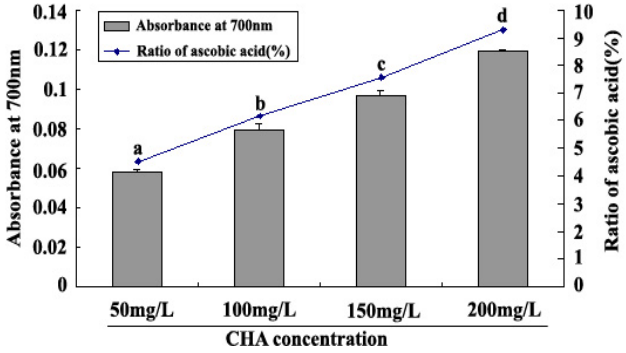

Figure 3. Reducing power ability of coffee husk anthocyanins (CHAs). Values are mean of triplicate ($n = 3$) with error bars indicating SD. Means with different lowercases indicate significant differences ($p < 0.05$).

Effects of coffee husk anthocyanins on levels of superoxide dismutase, lactate acid dehydrogenase, and malondialdehyde

Previous research showed that plant-based anthocyanins can protect cells against oxidative damage by regulating the activities of SOD and LDH, as well as the concentrations of MDA (Lee *et al.,* 2018; Wang *et al.,* 2018). Figure 5 shows the effects of CHAs on SOD and LDH activities, as well as the concentration of MDA in H_2O_2 oxidation stress-induced HUVEC cells. These results demonstrated that CHAs could increase the activity of SOD and suppress MDA and LDH (indicators of oxidative injuries). When the HUVEC cells were

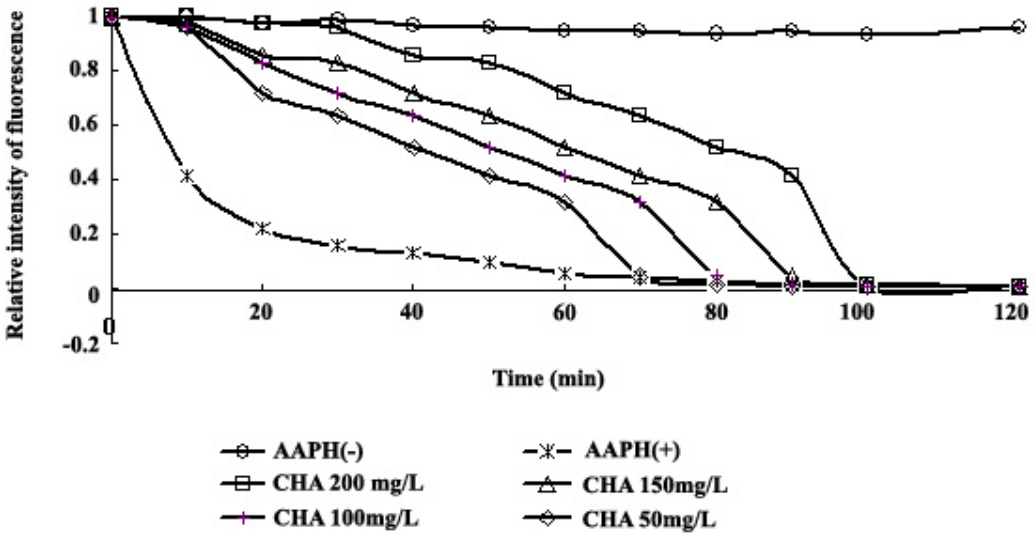

Figure 4. Oxygen radical absorbance capacity of coffee husk anthocyanins (CHAs). Values are mean of triplicate ($n = 3$) with error bars indicating SD.

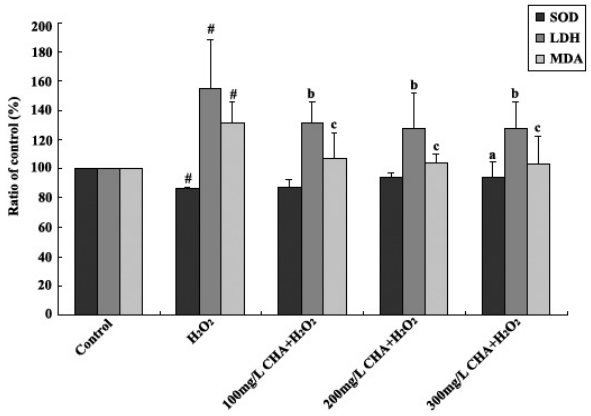

Figure 5. Effects of coffee husk anthocyanins (CHAs) treatment on the activities of SOD and LDH, as well as the MDA concentration in the culture supernatants and cell lysates of human umbilical vein endothelial (HUVEC) cells. Values are mean of six replicate (n = 6) with error bars indicating SD. Means with different lowercases indicate significant differences ($p < 0.05$). # indicates significant differences from control.

pre-treated with CHAs at 100, 200, or 300 mg/L (and were subsequently treated with H_2O_2), the percentages of activity of SOD increased by 87.78 ± 4.48, 93.97 ± 3.51, and 94.50 ± 10.28%, respectively, when compared with the control group. This indicated that CHAs enhanced the anti-oxidative capability of cells by increasing SOD activities. At the same time, the LDH percentage level of the H_2O_2 treatment group increased by 154.61 ± 33.51%, when compared with the control ($p < 0.05$). However, when HUVEC cells were pre-treated with CHAs at 100, 200, or 300 mg/L, (and were subsequently treated with H_2O_2), the LDH levels significantly decreased by 131.91 ± 14.27, 128.1 ± 24.31, and 127.86 ± 17.97%, ($p < 0.05$), respectively, when compared with the control group. In addition, the effect of CHAs on the MDA concentration was similar to that on LDH activities. The MDA percentage reached 130.83% in the H_2O_2 treatment alone when compared with the control group ($p < 0.05$). In response to increasing CHAs concentrations (100, 200, and 300 mg/L), the percentages of MDA concentration decreased in a dose-dependent manner to 106.67 ± 17.71, 104.17 ± 5.89, and 103.33 ± 18.86%, respectively, when compared with the control group. Cells treated with 300 mg/L CHAs showed significant differences when compared with the H_2O_2 treatment alone group. This dose-dependent decrease suggested that CHAs reduced the lipid-membrane oxidation by scavenging free radicals.

The SOD, the preferred detoxification enzyme and the most powerful antioxidant in the cell, is an important endogenous antioxidant enzyme that acts as a component of the first-line defence system against reactive oxygen species (Ighodaro and Akinloye, 2018). The SOD eliminates superoxide anion radicals, thus preventing oxidative damage in living cells. The CHAs could improve the antioxidative capability of cells by increasing their SOD activities in a dose-dependent manner. The MDA forms as one of the by-products of lipid peroxidation and can thus be used as a lipid peroxidation indicator, and is indirectly involved in the degree of cellular damage (Najeeb *et al.,* 2012). In the present work, the concentration of MDA significantly increased in the H_2O_2 treatment group, when compared with the non-treatment control group ($p < 0.05$). The contents of MDA decreased in the groups treated with different CHA concentrations, which might be related to the free radicals scavenging capability of cyanidin 3-rutinoside and cyanidin 3-glucoside (the main anthocyanins in CHs), thus interrupting lipid peroxidation. In addition, LDH assay was used to evaluate the cell membrane integrity and the extent of cell damage or death. The LDH levels of the cellular supernatant correlated with the oxidative injury of endothelial cells (Stancel *et al.,* 2016). Our previous work showed that the LDH levels of ECV340 cells significantly increased after H_2O_2 treatment, which increased cell death (Fan *et al.,* 2013). In the present work, the LDH levels decreased in the three groups treated with different concentrations of CHAs, when compared with the H_2O_2-treated group. This suggested that CHAs could maintain cell membrane integrity and prevent LDH leakage from cells.

Effect of inducing apoptosis on Caco-2 cells by coffee husk anthocyanins

Our previous results of MTT assay indicated that CHAs inhibited the growth of Caco-2 cells in a dose-dependent manner (data no shown). The result of annexin V-FITC combined with PI, for quantitatively assessing CHAs-induced apoptosis, demonstrated that CHAs induced Caco-2 cell apoptosis in a dose-dependent manner (Figure 6). The proportion of apoptotic Caco-2 cells significantly increased from 4.12 to 41.3%. Thus, CHAs induced apoptosis in Caco-2 cells, which resulted in the subsequent inhibition of cellular proliferation. The apoptosis induction on Caco-2 cells by CHAs may be caused by CHAs, cyanidin-3-glucoside, or cyanidin-3-rutinoside, which have been shown to have anti-cancer properties in previous studies. For example, the cyanidin-3-rutinoside and cyanidin-3-glucoside from açaí palm (*Euterpe*

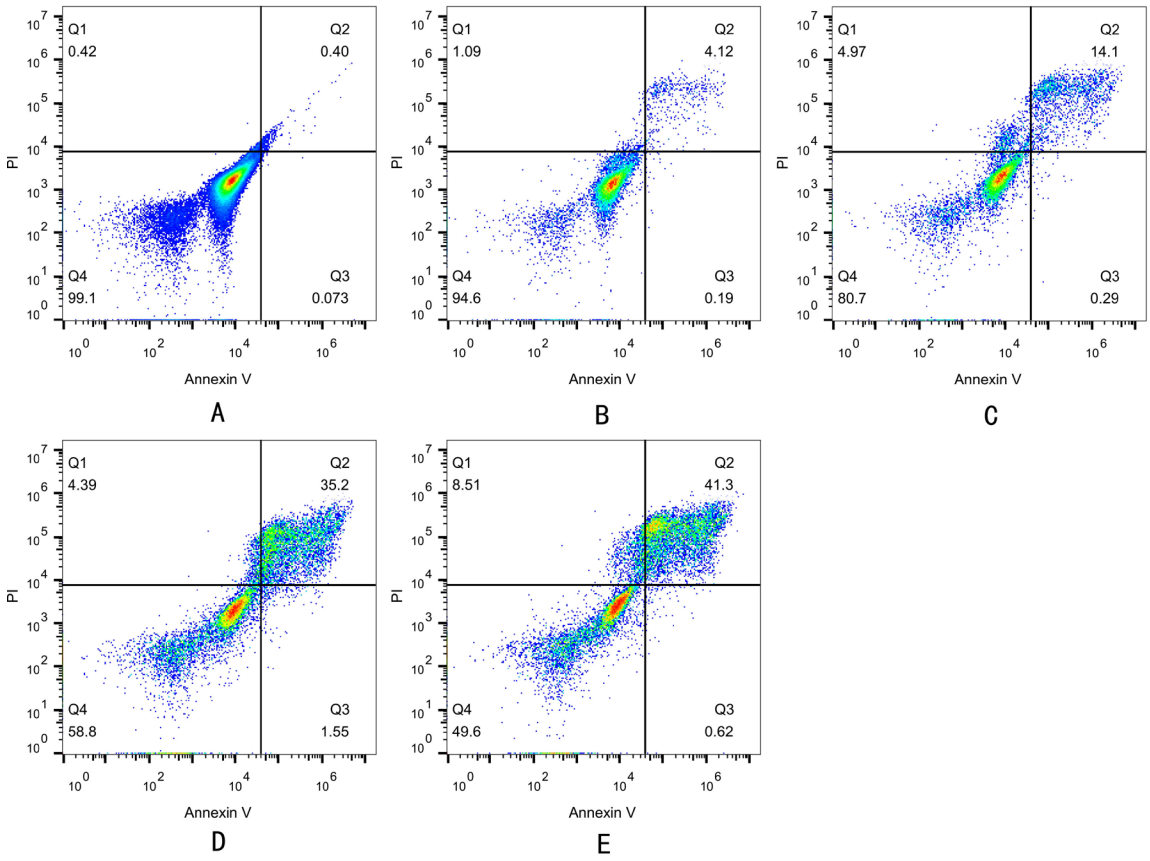

Figure 6. Flow cytometric analysis of Caco-2 cells treated with different concentrations of coffee husk anthocyanins (CHAs) for 24 h. A: Control group, B-E: CHA treatment groups (exposure to 50, 100, 150, and 200 mg/L extract, respectively).

oleracea Mart.) inhibited HT-29 colon cancer cell proliferation by up to 95.2%. Cyanidin 3-rutinoside and cyanidin 3-glucoside from mulberry (*Morus alba* L.) caused a stepwise suppression of cancer cell metastasis by significantly inhibiting the invasion of highly metastatic A549 cells (Chen *et al.,* 2006).

Conclusion

Based on the HPLC and LC-MS results, cyanidin-3-glucoside and cyanidin-3-rutinoside were identified as the main anthocyanins of CHs. CHAs showed strong antioxidant abilities in assays assessing their hydroxyl radical scavenging capacity, reducing power, and oxygen radical absorbance capacity. Moreover, the activities of SOD and LDH, as well as the concentration of MDA (*i.e.,* indicator for oxidative injuries in HUVEC cells exposed to H_2O_2 and treated with CHAs) showed that CHAs could reduce the oxidative damage of cells. CHAs induced apoptosis in Caco-2 cells in a concentration-dependent manner. Overall, these results confirmed that CHs can be used as functional compounds for food manufacturing.

Acknowledgement

The present work was financially supported by the National Natural Science Foundation of China-Yunnan Joint Fund (grant no.: U1902206), and the Special Program for Key Basic Research of Yunnan Provincial Education Department (grant no.: ZD2014007).

References

Amaia, I. D., Natalia, A. G., Beatriz, F. G., Eduardo, G. B. and María, D. C. 2019. Validation of coffee by-products as novel food ingredients. Innovative Food Science and Emerging Technologies 51: 194-204.

Anthony, K. P. and Saleh, M. A. 2013. Free radical scavenging and antioxidant activities of silymarin components. Antioxidants 2(4): 398-407.

Brendan, J. and Tien, H. 2018. Chemical composition and value-adding applications of coffee industry by-products: a review. Resources, Conservation and Recycling 128: 110-117.

Cagliani, L. R., Pellegrino, G., Giugno, G. and

Consonni, R. 2013. Quantification of *Coffea arabica* and *Coffea canephora* var. robusta in roasted and ground coffee blends. Talanta 106: 169-173.

Chen, P. N., Chu, S. C., Chiou, H. L., Kuo, W. H., Chiang, C. L. and Hsieh, Y. S. 2006. Mulberry anthocyanins, cyanidin 3-rutinoside and cyanidin 3-glucoside exhibited an inhibitory effect on the migration and invasion of a human lung cancer cell line. Cancer Letters 235(2): 248-59.

Chen, Q. L., Zhu, L., Tang, Y. N., Kwan, H. Y., Zhao, Z. Z., Chen, H. B. and Yi, T. 2017. Comparative evaluation of chemical profiles of three representative 'snow lotus' herbs by UPLC-DAD-QTOF-MS combined with principal component and hierarchical cluster analyses. Drug Testing and Analysis 9(8): 1105-1115.

De Melo Pereira, G. V., De Carvalho, N. D. P., Magalhaes Junior, A. I., Do Prado, F. G., Pagnoncelli, M. G. B., Karp, S. G. and Soccol, C. R. 2020. Chemical composition and health properties of coffee and coffee by-products. Advances in Food and Nutrition Research 91: 65-96.

Duangjai, A., Suphrom, N., Wungrath, J., Ontawong, A., Nuengchamnong, N. and Yosboonruang, A. 2016. Comparison of antioxidant, antimicrobial activities and chemical profiles of three coffee (*Coffea arabica* L.) pulp aqueous extracts. Integrative Medicine Research 5(4): 324-331.

Fan, J. P., Fan, C., Dong, W. M., Gao, B., Yuan, W. and Gong, J. S. 2013. Free radical scavenging and anti-oxidative activities of an ethanol-soluble pigment extract prepared from fermented Zijuan Pu-erh tea. Food and Chemical Toxicology 59: 527-533.

Gabriela, L. A., Julio, M. A., Leticia, S. X., Sylvia, P. D. C., Valentin, M. S., Jose, A. L. V. and Francisco, D. V. 2018. Anthocyanins of *Pithecellobium dulce* (Roxb.) Benth. Fruit associated with high antioxidant and α-glucosidase inhibitory activities. Plant Foods for Human Nutrition 73(4): 308-313.

Galvano, F., La, F. L., Vitaglione, P., Fogliano, V., Vanella, L. and Felgines, C. 2007. Bioavailability, antioxidant and biological properties of the natural free-radical scavengers cyanidin and related glycosides. Annali dell'Istituto Superiore di Sanità 43(4): 382-393.

Ighodaro, O. M. and Akinloye, O. A. 2018. First line defence antioxidants-superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX): their fundamental role in the entire antioxidant defence grid. Alexandria Journal of Medicine 54: 287-293.

Je, J. Y., Ahn, C. B., Oh, M. J. and Kang, S. Y. 2009. Antioxidant activity of a red seaweed *Polysiphonia morrowii* extract. Food Science Biotechnology 18: 124-129.

Klingel, T., Kremer, J. I., Gottstein, V., Rezende, T. R., Schwarz, S. and Lachenmeier, D. W. 2020. A review of coffee by-products including leaf, flower, cherry, husk, silver skin, and spent grounds s novel foods within the European Union. Foods 9(5): article no. 665.

Krga, I. and Milenkovic, D. 2019. Anthocyanins: from sources and bioavailability to cardiovascular-health benefits and molecular mechanisms of action. Journal of Agricultural and Food Chemistry 67(7): 1771-1783.

Lee, Y. S., Cho, I. J., Kim, J. W., Lee, S. K., Ku, S. K. and Lee, H. J. 2018. Evaluation of *in vitro* antioxidant and anti-inflammatory activities of Korean and Chinese *Lonicera caerulea*. Nutrition Research and Practice 12(6): 486-493.

Mellado-Ortega, E., Zabalgogeazcoa, I., Vázquez de Aldana, B. R. and Arellano, J. B. 2017. Solutions to decrease a systematic error related to AAPH addition in the fluorescence-based ORAC assay. Analytical Biochemistry 519: 27-29.

Moreira, M. D., Melo, M. M., Coimbra, J. M., Reis, K. C. D., Schwan, R. F. and Silva, C. F. 2018. Solid coffee waste as alternative to produce carotenoids with antioxidant and antimicrobial activities. Waste Manager 82: 93-99.

Murthy, P. S. and Madhava, N. M. 2012. Sustainable management of coffee industry by-products and value addition - a review. Resources, Conservation and Recycling 66: 45-58.

Najeeb, Q., Bhaskar, N., Masood, I., Wadhwa, S., Kaur, H. and Ishaq, S. 2012. Malondialdehyde (MDA) Superoxide dismutase (SOD) levels-distinguishing parameters between benign malignant pleural effusions. Free Radical Antioxidants 2: 8-11.

Oliveira, L. S. and Franca, A. S. 2015. An overview of the potential uses for coffee husks. In Preedy, V. R. (ed). Coffee in Health and Disease Prevention, p. 283-291. United States: Academic Press.

Orhan, I., Kartal, M., Abu-Asaker, M., Şenol, F. S., Yilmaz, G. and Şener, B. 2009. Free radical scavenging properties and phenolic characterization of some edible plants. Food Chemistry 114(1): 276-281.

Pavithra, K. and Sasikumar, V. 2015. Evaluation of free radical scavenging activity of various

extracts of leaves from *Kedrostis foetidissima* (Jacq.) Cogn. Food Science Human Wellness 4: 42-46.

Prata, E. R. and Oliveira, L. S. 2007. Fresh coffee husks as potential sources of anthocyanins. LWT - Food Science and Technology 40: 1555-1560.

Prior, R. L. and Wu, X. 2006. Anthocyanins: structural characteristics that result in unique metabolic patterns and biological activities. Free Radical Research 40: 1014-1028.

Shi, X. W., Zhou, N., Cheng, J. Y., Shi, X. L., Huang, H., Zhou, M. M. and Zhu, H. Y. 2019. Chlorogenic acid protects PC12 cells against corticosterone-induced neurotoxicity related to inhibition of autophagy and apoptosis. BMC Pharmacology Toxicology 20(1): article no. 56.

Stancel, N., Chen, C. C., Ke, L. Y., Chu, C. S., Lu, J., Sawamura, T. and Chen, C. H. 2016. Interplay between CRP, atherogenic LDL, and LOX-1 and its potential role in the pathogenesis of atherosclerosis. Clinical Chemistry 62(2): 320-327.

Steed, L. E. and Truong, V. D. 2008. Anthocyanin content, antioxidant activity, and selected physical properties of flowable purple-fleshed sweet potato purees. Journal of Food Science 73: S215-S221.

Szymanowska, U., Baraniak, B. and Bogucka-Kocka, A. 2018. Antioxidant, anti-inflammatory, and postulated cytotoxic activity of phenolic and anthocyanin-rich fractions from Polana raspberry (*Rubus idaeus* L.) fruit and juice - *in vitro* study. Molecules 23(7): article no. 1812.

Tores de la Cruz, S., Iriondo-DeHond, A., Herrera, T., Lopez-Tofiño, Y., Galvez-Robleño, C., Prodanov, M., … and Castillo, M. 2019. An assessment of the bioactivity of coffee silverskin melanoidins. Foods 8(2): article no. 68.

Wang, Z., Gu, D., Sheng, L. and Cai, J. 2018. Protective effect of anthocyanin on paraquat-induced apoptosis and epithelial- mesenchymal transition in alveolar type II cells. Medical Science Monitor 24: 7980-7987.

Wei, F. F., Furihata, K., Koda, M., Hu, F. Y., Miyakawa, T. and Tanokura, M. 2012. Roasting process of coffee beans as studied by nuclear magnetic resonance: time course of changes in composition. Journal of Agricultural and Food Chemistry 60(4): 1005-1012.

Wenzel, U., Kuntz, S., Brende, l. M. D. and Daniel, H. 2000. Dietary flavone is a potent apoptosis inducer in human colon carcinoma cells. Cancer Research 60: 3823-3831.

Yi, T., Chen, Q. L., He, X. C., So, S. W., Lo, Y. L., Fan, L. L., ... and Chen, H. B. 2013. Chemical quantification and antioxidant assay of four active components in *Ficus hirta* root using UPLC-PAD-MS fingerprinting combined with cluster analysis. Chemistry Central Journal 7: article no. 115.

Zeng, C. Z., Liu, Z. X., Wu, Y. X. and Wang, X. L. 2007. Studies on flavonoids extraction by ultrasonic technology from *Glycyrrhiza* and their bacteriostatic activity. Lishizhen Medicine and Materia Medica Research 18: 2402-2403.

Zhao, C., Qiao, X. L., Shao, Q. J., Hassan, M. and Ma, Z. Q. 2020. Evolution of lignin chemical structure during bioethanol production process and its inhibition to enzymatic hydrolysis. Energy Fuels 34(5): 5938-5947.

Zhu, L., Han, Q. B., Ho, A., Hsiao, W. L. and Jiang, Z. H. 2013. Characterization and simultaneous determination of immunosuppressive decalins in red yeast rice by ultra-high-performance liquid chromatography hyphenated with mass spectrometry. Journal of Chromatography A 1303: 54-61.

Zhu, M., Huang, Y., Wang, Y., Shi, T., Zhang, L., Chen, Y. and Xie, M. 2019. Comparison of (poly) phenolic compounds and antioxidant properties of pomace extracts from kiwi and grape juice. Food Chemistry 271: 425-432.