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ULTRASOUND-ASSISTED EXTRACTION AND ANTICANCER ACTIVITY OF PICEATANNOL FROM *Passiflora edulis* SEED

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ABSTRACT

Ultrasound-assisted extraction of piceatannol from *Passiflora edulis* seeds was studied. Effects of ethanol concentration, temperature, and ultrasonic time were investigated. The best extraction conditions were as follows: ethanol concentration, 80% (*v/v*); temperature, 70°C; and extraction time, 30 min. Under the optimal conditions, the yield of piceatannol content was 4.5 ± 0.17 mg per gram of seed dry matter. The freeze-dried piceatannol extract powder exhibited anticancer activity against two cancer cell lines, HeLa and MCF7. This study should be considered as a first step for the production of piceatannol-rich products to be used as nutraceuticals from passion fruit seeds, a by-product of passion fruit juice production.

Keywords: Anticancer activity, *Passiflora edulis* seed, piceatannol, ultrasound-assisted extraction.

Tách chiết có hỗ trợ của siêu âm và hoạt động kháng ung thư của piceatannol từ hạt chanh leo (*Passiflora edulis)*

TÓM TẮT

Quá trình tách chiết piceatannol từ hạt chanh leo - *Passiflora edulis* có hỗ trợ của siêu âm được nghiên cứu. Ảnh hưởng của nồng độ ethanol, nhiệt độ và thời gian siêu âm đến hàm lượng picetannol tách chiết được khảo sát. Điều kiện tốt nhất cho sự tách chiết như sau: nồng độ ethanol, 80% (v/v); nhiệt độ 70°C; thời gian tách chiết, 30 phút. Với điều kiện này, hiệu suất thu hồi piceatannol là 4.5 ± 0.17 mg từ 1 g chất khô hạt. Bột đông khô dịch chiết piceatannol thể hiện khả năng kháng hai dòng tế bào ung thư bao gồm HeLa và MCF7. Nghiên cứu này có thể coi như bước đầu cho việc sản xuất sản phẩm giàu piceatannol sử dụng như sản phẩm hỗ trợ sức khỏe từ hạt chanh leo, một phụ phẩm của quá trình chế biến nước quả.

Từ khóa: Chiết có hỗ trợ của siêu âm, hạt *Passiflora edulis*, hoạt động kháng ung thư, piceatannol.

1. INTRODUCTION

Passion fruit (*Passiflora edulis* Sims) belongs to the Passifloraceae family and is native to South America from southern Brazil through Paraguay to northern Argentina (Morton, 1987). The fruit has appealing flavor and presents many health benefits. According to the USDA National Nutrient Database (https://ndb.nal.usda.gov/ndb/foods/show/2308?manu=&fgcd=), one serving portion of passion fruit (236 g) provided 98%, 118%, 60%, 23%, and 21% of the recommended daily intake for humans of dietary fiber, vitamin C, vitamin A, potassium, and iron, respectively. Besides, passion fruit contains many phytochemicals, such as cyanidin 3-glucoside, cyanidin 3-(6"-malonylglucoside), and pelargonidin 3-glucoside

in the rind (Kidoy *et al.*, 1997), and piceatannol in the seed (Matsui *et al.*, 2010), which are known as being beneficial for human health. For example, piceatannol has been shown to have potent biological activities, including antioxidant (Ovesná *et al.*, 2006), anti-cancer (Vo *et al.*, 2010; Kita *et al.*, 2012), anti-inflammatory (Son *et al.*, 2010), and anti-obesity properties (Kwon *et al.*, 2012). Interestingly, passion fruit seeds have a very high piceatannol concentration of 2.2 mg/g dry weight (DW), which is 4,000 times higher than the concentration in red grapes (Guerrero *et al.*, 2010), a major source of piceatannol in the human diet.

Cultivation of passion fruit is increasing in Vietnam. The fruits are used to make both fresh juice and concentrated juice. These processes generate rinds and seeds as by-products, and make up approximately 40% and 12% of the starting materials, respectively (Matsui *et al.*, 2010). Hence, thousands of tons of passion fruit seeds will be discharged each year by food factories. This by-product may be used to extract piceatannol and then to produce picetannol-rich products that can be further used in food and drug technologies.

Bioactive compounds, in general, and piceatannol, in particular, can be extracted by conventional or non-conventional methods. Conventional extraction is usually performed using maceration, reflux, soxhlet, or hydrodistillation. These methods are very time consuming and require relatively large quantities of solvents. Extraction using non-conventional methods, such as ultrasound assisted extraction, can result in a yield increase in a shorter amount of time (a few minutes compared to several hours in conventional methods) using less solvent (Bandar *et al.*, 2013). Indeed, the beneficial effects of ultrasonic extraction are attributed to the formation and asymmetrical collapse of microcavities in the vicinity of cell walls leading to the generation of microjets rupturing the cells in plant tissues (Zhou *et al.*, 2011), and to the enhancement of compound diffusion from the matrix into the solvent (Chen *et al.*, 2014). This technique has successfully been used to extract phenolic compounds from areca husks (Wang *et al.*, 2013; Chen *et al.*, 2014), antioxidant compounds from *Morus alba* L. (Thong *et al.*, 2014), and flavonoids from *Eriobotrya japonica* Lindl. flowers (Zhou *et al.*, 2011).

The present study had two purposes: the first was to optimize the ultrasound-assisted extraction parameters of piceatannol from passion fruit seeds, and the second was to investigate the anticancer activity of the freeze-dried piceatannol extract powder against three cancer cell lines.

2. MATERIALS AND METHODS

2.1. Sample collection and preparation

The passion fruit seeds (*Passiflora edulis*) were collected from Nafoods Group (Nghe An, Vietnam) in September 2013. They were by-products of the production of passion fruit concentrated juice. Approximately 20 kg of fresh seeds were collected and transported to the laboratory on the day of production. The seeds were first washed with tap water to remove the membranes around the seeds. The seeds were then rinsed in distilled water three times and dried under sunlight. The dried seeds were ground using a TecatorCyclotec 1093 sample mill (Sweden), kept in a sealed plastic bag, and stored at -53ºC until extraction.

For the production of piceatannol extract powder used in the anticancer tests, piceatannol in the passion fruit seeds was extracted using 80% ethanol (*v/v*) at 70ºC for 30 minutes and with the assistance of ultrasound of 37 kHz/600W. The extracted solution was then centrifuged at 6,000 rpm for 10 min at 4ºC (Mikro 220R, Hettichzentrifugen, Germany). The supernatant was concentrated in a rotatory evaporator (Buchilabortechnik AG, Switzerland) under reduced pressure at a temperature of 40ºC, and dried in a lyophilizer (FR-Drying Digital unit-Thermo, MA). The lyophilized extract powder was stored at 4ºC for further anticancer tests.

2.2. Chemicals and reagents

The piceatannol standard, ethylenediaminetetraacetic acid (EDTA), dihydroethidium (DHE), and propidium iodide were purchased from Sigma-Aldrich (St. Louis, MO). Analytical grade ethanol, and HPLC grade acetonitrile and acetic acid were obtained from Merck (Darmstadt, Germany).

2.3. Ultrasound-assisted extraction of piceatannol from *Passiflora edulis* seed

Ultrasound-assisted extraction was performed in an ultrasonic cleaning bath (Elma S60H, Germany) with a useful volume of 6 L. Working frequency and power were fixed at 37 kHz and 600 W, respectively. Approximately 0.25 g of powdered, dried sample was mixed with 5 mL of solvent (ethanol at different concentrations) in a 15 mL Falcon conical centrifuge tube. The tube then was placed in the bath and sonicated for different times at the required temperatures. After centrifugation at 3,642 *g* (6,000 rpm) for 10 min at 4°C, the supernatant was collected. The solution was filtered through a 0.42 m syringe filter (Phenex™-NY, Utrecht, The Netherlands) before analysis by HPLC-UV/VIS. Each extraction was done in triplicate.

2.3.1. Effect of ethanol concentration on extraction of piceatannol

Ethanol in water was used as the extraction solvent. Piceatannol from the passion fruit seeds was extracted using various ethanol concentrations, ranging from 20 to 99.5% (absolute) (*v/v*). Dried passion fruit seed powder (0.25 g) was steeped in the extracting solvent (5 mL), and sonicated for 30 min at 50°C. The extract was centrifuged at 3,642 *g* (6,000 rpm) for 10 min at 4°C. The supernatant was collected and the piceatannol content analyzed.

2.3.2. Effect of extraction temperature on extraction of piceatannol

Dried passion fruit seed powder (0.25 g) was mixed with 5 mL of optimal extraction ethanol concentration and sonicated for 30 min at different temperatures (30 to 70°C). The mixture was then centrifuged at 3,642 g (6,000 rpm) for 10 min at 4°C. The piceatannol content of the supernatant was analyzed.

2.3.3. Effect of extraction time on extraction of piceatannol

Dried passion fruit seed powder (0.25 g) was mixed with 5 mL of optimal extraction ethanol concentration and sonicated for various times ranging from 5 to 120 min at the optimal extraction temperature. The mixture was centrifuged at 3,642 *g* (6,000 rpm) for 10 min at 4°C. The supernatant was collected and the piceatannol content analyzed.

2.4. Determination of piceatannol by HPLC

Quantification of picetananol in the extract was performed by HPLC using a Shimadzu system (Japan) equipped with a LC-10Ai pump, a DGU-20A3 degasser, a SPD-20A UV/VIS detector, and a CBM-20A interface. A 20 L aliquot of the piceatannol extract was manually injected into a reversed-phase C18 column (ODS) (100 × 3 mm i.d.; 5 μm particle size) equipped with a guard column of the same type (Agilent, CA). The mobile phases were A (20 μg/mL EDTA, 2% acid acetic, 9% acetonitrile) and B (20 μg/mL EDTA, 2% acid acetic, 80% acetonitrile). The flow rate was 1 mL/min, and the column temperature was set at 35°C. The 32 min gradient was as follows: 0 - 4 min, 0% B; 4 - 8 min, 0 - 35% B; 8 - 18 min, 35 - 80% B; 18 - 20 min, 80 - 100% B; 20 - 25 min, 100% B; 25 - 30 min, 100 - 0% B; and 30 - 32 min, 0% B. The monitoring system was set at 320 nm for quantification of piceatannol. Piceatannol in the extract was identified by its retention time as compared to the authentic standard, and was quantified using five-point calibration curves ($y = 10{,}034x - 807.9$; $R^2 = 0.999$).

2.5. Anticancer activity analyses

2.5.1. Analysis of reactive oxygen species (ROS), cell cycle arrest, and apoptosis

Analyses of reactive oxygen species (ROS), cell cycle arrest, and apoptosis were done as described by Kitanovic *et al.* (2009). Three

human Panc1 (pancreatic carcinoma), MCF7 (breast adenocarcinoma) and HeLa (cervical carcinoma) cell lines were purchased from Sigma-Aldrich (Germany). Cancer cells were plated in 12-well plates at a density of 200,000 cells/well and cultivated under standard conditions for 24 h before cells were treated with piceatannol extract as described in the text. Cells were collected by trypsinization and centrifugation at 200 g (1500 rpm), and resuspended in 2 mL of FACS (fluorescence activated cell sorting) buffer (1% BSA in phosphate buffered saline (PBS)). For ROS determination, the cell suspension was supplemented with 5 μM dihydroethidium. After 15 min of incubation at room temperature in the dark, cells were washed with FACS buffer. For the cell cycle arrest analysis, the cell suspension was incubated with RNase A (50 μg/mL) for 30 min at 37°C, and sequentially stained with propidium iodide (PI, 50 μg/mL) for 1 h and analysed by FACS (fluorescence activated cell sorting). At least two independent experiments were performed. After staining with specific chemicals and incubation, all aliquots of cell suspensions were immediately analysed using a FACSCalibur flow-cytometer (Becton Dickinson) and CellQuest Pro (BD) analysis software.

2.5.2. Cell cytotoxicity assay

The effects of the piceatannol extract powder (0.02 mg/mL) on cell growth were determined using the 3-(4,5-dimethylthiazolyl-2)-2,5-diphenyltetrazolium bromide assay (MTT assay, ATCC company, Germany) at an initial cell density of 5,000 cells/well in a 96-well plate. The protocol was performed according to the instructions of the manufacture (ATCC).

2.5.3. Wound healing assay

The wound healing assay was done according to protocol of Cheng *et al.* (2014). Cancer cell lines (Panc1, MCF7, and HeLa) were plated at high density (200,000 cells/well) into 12-well plates and grown to confluence. The scratch was made by a sterile P-200 micropipette in the middle of each well. Cell suspensions were then washed three times with PBS buffer and treated with 0.02 mg/mL of the piceatannol extract powder. Photographs were taken after two days of incubation at 37°C.

2.6. Statistical analysis

All extractions were performed in triplicate. The apparent contents of piceatannol obtained under different conditions were analysed by the SAS 9.0 software (SAS Institute, Cary, NC) and expressed as mean ± standard deviation. One way analysis of variance (ANOVA) and Duncan's test were used to determine the differences amongst the means. P-values < 0.05 were considered to be significantly different.

3. RESULTS AND DISCUSSION

3.1. Ultrasound-assisted extraction of piceatannol from *Passiflora edulis* seed

3.1.1. Effect of ethanol concentration on extraction of piceatannol

Water-ethanol mixtures were used as the extraction solvents in this study. The selection of ethanol as the extraction solvent was justified by the fact that ethanol is a food grade solvent, is less toxic, and is more abundant as compared to acetone, methanol, and other organic solvents (Kiassos *et al.*, 2009; Chew *et al.*, 2011). The use of ethanol at different concentrations in water was chosen because binary-solvent systems have demonstrated higher yields of phenolic compounds when compared to mono-solvent systems (Zhou *et al.*, 2011; Wang *et al.*, 2013; Lai *et al.*, 2014). In this study, ethanol concentration showed significant effects on apparent piceatannol content ($p < 0.0001$). Indeed, the apparent piceatannol content mounted up with an increase in ethanol concentration, reached its highest value (2.26 ± 0.01 mg/g DW) at 80% ethanol, and then began to decrease (Figure 1). This result is in accordance with the results of Matsui *et al.* (2012), who reported that extractions with 80% aqueous ethanol provided the highest efficiency for piceatannol extraction from passion fruit seeds (about 50 mg/100 g of freeze-dried seed powder).

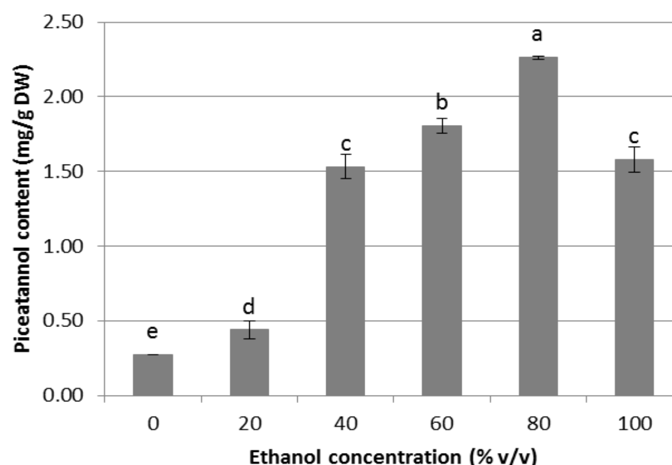

Figure 1. Effect of ethanol extraction concentration on the apparent piceatannol content of passion fruit seeds

Note: Values marked by the same letter are not significantly different (p < 0.05). Ultrasound-assisted extraction conditions: extraction temperature, 50°C; extraction time, 30 min; ultrasound frequency and power, 37 kHz and 600 W.

The effects of ethanol concentration in the extraction medium on phenolic compounds (in general) and on piceatannol (in particular) yields have been observed in various studies. Lai *et al.* (2014) found that the ethanol concentration was the most affecting factor in the extraction of piceatannol from sim seeds (*Rhodomyrtus tomentosa*) with 79% as the optimal value. The best ethanol concentrations for the ultrasound-assisted extraction of phenolic compounds from areca husks (*Areca catechu* L.) and from loquat (*Eriobotrya japonica* Lindl.) flowers were 41% and 60%, respectively (Zhou *et al.*, 2011; Chen *et al.*, 2014). The impact of ethanol concentration is due to its effect on the polarity of the extraction solvent and the resulting solubility of the phenolic compounds. The general principle is "like dissolves like," which means that solvents only extract those phytochemicals that have a similar polarity to that of the solvent (Lai *et al.*, 2014). Since the highest apparent picetannol content reached a maximum when ethanol concentration was of 80%, this concentration was chosen and used in further extractions.

3.1.2. Effect of extraction temperature on extraction of piceatannol

Figure 2 shows the effect of extraction temperature under sonication on the apparent content of piceatannol. Temperature had a significant effect on piceatannol extraction from passion fruit seeds ($p < 0.0001$). An increase in the apparent piceatannol content was observed over the extraction temperature range (30 - 70°C). This effect of temperature was in accordance with studies on piceatannol extraction from *Rhodomyrtus tomentosa* seeds (Lai *et al.*, 2014), and on phenolic extraction from areca husks (Chen *et al.*, 2012). An increase in the extraction temperature may increase the solubility of piceatannol in the solvent and decrease the viscosity of the solvent. The combination of these two phenomena enhanced the overall extraction efficiency (Chen *et al.*, 2012). However, the phenolic yield, after having a high value, decreased when the extraction temperature increased due a possible concurrent decomposition of phenolic compounds. In the work of Lai *et al.* (2014), piceatannol yield from sim seeds reached a maximum at 85°C and then decreased. Zhou *et al.* (2011) had highest phenolic and flavonoid contents at 50°C, while Chen *et al.* (2012) had a maximum phenolic concentration at 75°C during the ultrasound-assisted extraction from loquat flowers and areca husks, respectively. In this study, because of the low capacity of the ultrasonic cleaning bath, the extraction temperature could not be higher than 70°C. As

the highest apparent piceatannol content of the passion seed was obtained at 70°C, this temperature was chosen for the piceatannol extraction with the assistance of ultrasound.

3.1.3. Effect of extraction time on extraction of piceatannol

The amount of piceatannol extracted from passion fruit seeds as a function of sonication time are presented in Figure 3. Apparent piceatannol content of the seeds increased markedly during the first 30 min with a rate of 0.10 - 0.15 mg/g DW per minute and then seemed stablize. This result agreed with other research on phenolic extractions from plant materials. Indeed, Chen *et al.* (2012) found that total phenolics extracted from areca husks increased markedly up to 30 min, then remained constant at 40 min. Ultrasonic extraction of flavonoids and phenolics from loquat flowers showed that the extraction rate became slow after 80 min (Zhou *et al.*, 2011). Alternately, a high concentration of piceatannol (5.82 mg/DW) from *Rhodomyrtus tomentosa* seeds was observed when the time of extraction was only 4.6 min. When the extraction time increased from 4.6 to 55 min, the apparent piceatannol content increased very slightly from 5.82 to 6.27 mg/g DW (Lai *et al.*, 2014). In this study, an extraction time of 30 minutes was chosen for the sake of saving time and energy.

Based on our results, optimal piceatannol extraction conditions from passion fruit seeds with assisted sonication were as follows: ethanol concentration, 80% (*v/v*); extraction temperature, 70°C; ultrasound frequency and power, 37 kHz and 600W; and extraction time, 30 minutes. Under these conditions, 4.5 ± 0.17 mg of piceatannol was obtained from one gram of dried passion fruit seeds. In comparison to the results of Matsui *et al.* (2012), who did not use the ultrasound, our piceatannol yield was 2 - 9 times higher. Ultrasound exerts a mechanical effect, effecting greater penetration of solvent into the sample matrix, and increasing the contact surface area between the solid and liquid phases; as a result, the solute quickly diffuses from the solid phase to the solvent. This is a really good technique in the exploitation of bioactive compounds.

3.2. Anticancer activity of the piceatannol extract powder

3.2.1. ROS formation, cell cycle arrest, and cell cytotoxicity assays

High intracellular ROS levels pose a significant threat to cellular integrity, and can lead to mitochondrial DNA damage and subsequent induction of cell cycle arrest for DNA repair and for programmed cell death (apoptosis). Apoptosis plays a crucial role in the normal development of cells and in the inhibition of tumor growth and development (Kim *et al.*, 2011). Apoptosis can be induced by a variety of agents. Of which, there are various cytotoxic substances. The results, summarized in Table 1, showed that the piceatannol extract powder induced high intracellular ROS levels in two cancer cell lines, MCF7 and HeLa. The ROS levels generated by MCF7 and HeLa cells upon treatment with piceatannol extract powder were 4.0 ± 0.32 and 4.5 ± 0.28 times higher than that formed by untreated cancer cells, respectively. To cope with the damage of high ROS levels, cell cycle arrest was triggered at the G2/M phase before cell division, thereby inhibiting cancer cells from developing. Moreover, the anti-proliferative or cytotoxic effect of piceatannol extract powder on MCF7 and HeLa was also indicated by IC_{50} values of 6.2 ± 0.01 and 5.4 ± 0.02 µg/mL in the respective cell lines. In contrast, the cytotoxic effect of piceatannol extract powder was not detected in the Panc1 cell line, even at 50 µg/mL. This could be that Panc1 was insensitive or less sensitive to piceatannol extract powder when compared to MCF7 and HeLa. Thus, piceatannol extract powder did not induce a significantly high ROS level to induce cell cycle arrest (Table 1).

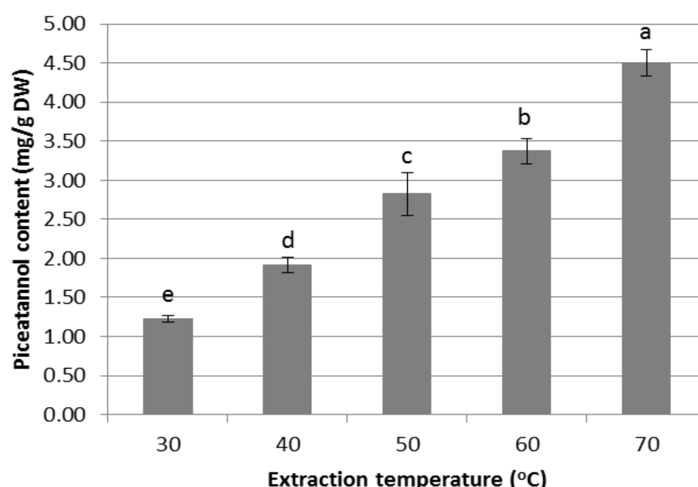

Figure 2. Effect of extraction temperature on the piceatannol content of passion fruit seeds

Note: Values marked by the same letter are not significantly different ($p < 0.05$). Ultrasound-assisted extraction conditions: ethanol concentration, 80% (v/v); extraction time, 30 min; ultrasound frequency and power, 37 kHz and 600 W.

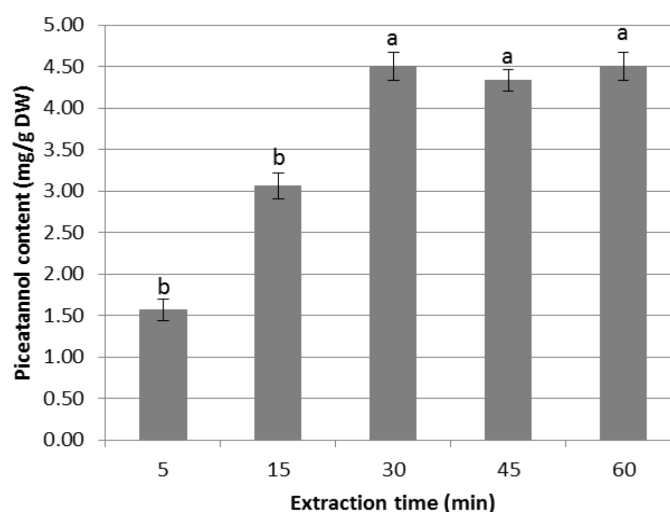

Figure 3. Effect of extraction time on the piceatannol content of passion fruit seeds

Note: Values marked by the same letter are not significantly different ($p < 0.05$). Ultrasound-assisted extraction conditions: ethanol concentration, 80% (v/v); extraction temperature, 70°C; ultrasound frequency and power, 37 kHz and 600 W.

Table 1. Anticancer activity of piceatannol in three human cancer cell lines

Cell line	Cytotoxicity* (IC_{50}, μg/ml)	ROS** (Fold)	Cell cycle arrest*** (Phase)
Panc1	> 50.0	1.2 ± 0.21	Not determined
MCF7	6.2 ± 0.01	4.0 ± 0.32	G2/M
HeLa	5.4 ± 0.02	4.5 ± 0.28	G2/M

*Note: *Half maximal inhibitory concentration (IC_{50}) of compound in inhibiting cell growth was calculated from dose-response curves in three independent experiments.*
***ROS, expressed by fluoresence intensity, was calculated by normalization of ROS level of treated cells to that of untreated cells (control) giving folds or times.*
****Cell cycle at which certain phases are arrested in the specific point of cell division cycle.*

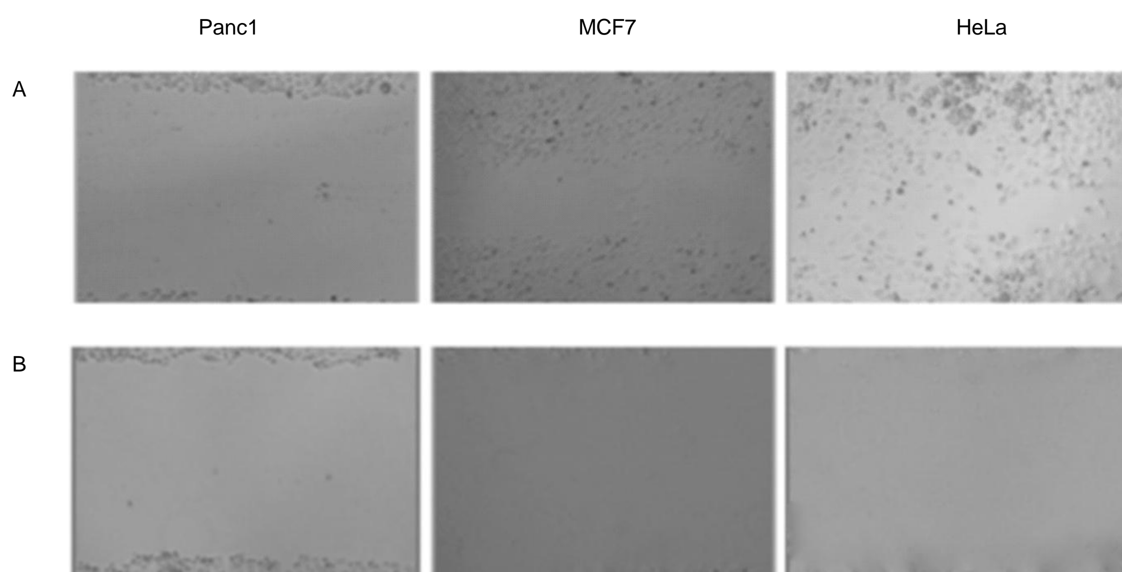

Figure 4. Inhibition of cell migration in untreated cells (control, above - A) and cells treated with piceatannol extract powder (below - B)

3.2.2. Wound healing assay

In addition, the anticancer activity of piceatannol extract powder was tested and confirmed by inhibition of migration (Figure 4). Indeed, treatment of MCF7 and HeLa cell lines with piceatannol extract powder led to very low cell proliferation. Only a few cells were able to enter the gap generated from a scratch in the cell layer, indicating reduced cell mobility in the presence of piceatannol extract powder. Nevertheless, migration of Panc1 cells seemed not to be affected in the presence of piceatannol extract powder (Figure 4).

In comparing the results from this study to those from other studies, the piceatannol extract was not effective in showing anticancer activity against Panc1 as compared to the gold(I)NHC complex (MC3) (Ewton *et al.*, 2011; Cheng *et al.*, 2014). The MC3 efficiently suppressed cell growth, and induced cell cycle arrest and apoptosis in Panc1 since it caused a substantial alteration of the cellular redox homeostasis leading to increased ROS levels and a decrease in the mitochondrial membrane potential (Cheng *et al.*, 2014).

Regarding the results of the piceatannol extract against MCF7, the findings obtained in this study agreed with those found in recently published reports stating that many compounds, including sythetic and natural compounds, such as flavopiridol, piperine, tetrahydrocurcumin, and phenolic compounds, were also effective in exhibiting anticancer activity against MCF7 (de Souza Grinevicius *et al.*, 2016; Han *et al.*, 2016; Kwan *et al.*, 2016; Li *et al.*, 2013; Shao *et al.*, 2016). Similar to these reports, the same effects, cytotoxicity, cell cycle arrest, high ROS level, and apoptosis, were observed when HeLa was exposed to a range of concentrations of ruthenium(II) complexes, quinolones, and daidzein (Han *et al.*, 2015; Kumar *et al.*, 2015; Jantova *et al.*, 2016; Zeng *et al.*, 2016).

However, to understand and evaluate the overall anticancer activity of piceatannol, this compound or its derivative will need to be tested with other main human cancer cell lines (UM-UC-10 (bladder), SVCT (breast), MDST8 (colon), etc.), thereby helping to elucidate the action mechanism of piceatannol. Additionally, in order to use piceatannol in functional food products or to develop pharmaceutical materials, further studies, such as bioavailability, bioequivalence, safety, tolerability, and clinical trials should be investigated.

4. CONCLUSIONS

Ultrasound enhanced piceatannol extraction from passion fruit seeds. Ultrasound-assisted extraction conditions were determined and were as follows: ethanol concentration, 80% (*v/v*); extraction temperature, 70°C; ultrasound frequency and power, 37 kHz and 600 W; and extraction time, 30 minutes. The freeze-dried piceatannol extract powder showed anticancer activity in two cancer cell lines, HeLa and MCF7. This study provided the first bases for the production of piceatannol-rich products to be used as nutraceuticals from one of the by-products of passion fruit food technology.

REFERENCES

Chen W., Y. Huang, J. Qi, M. Tang, Y. Zheng, S. Zhao, L. Chen (2014). Optimisation of ultrasound-assisted extraction of phenolic compounds from areca husk. J. Food Process. Pres., 38(1): 90-96.

Chew K. K., S. Y. Ng, Y. Y. Thoo, M. Z. Khoo, W. M. Wan Aida, C. W. Ho (2011). Effect of ethanol concentration, extraction time and extraction temperature on the recovery of phenolic compounds and antioxidant capacity of *Centella asiatica* extracts. Int. Food Res. J., 18(2): 571-578.

Cheng X., P. Holenya, S. Can, H. Alborzinia, R. Rubbiani, I. Ott, S. Wolfl (2014). A TrxR inhibiting gold (I) NHC complex induces apoptosis through ASK1-p38-MAPK signaling in pancreatic cancer cells. Molecular Cancer**,** 13: 221.

de Souza Grinevicius V. M., M. R. Kviecinski, N. S. Santos Mota, F. Ourique, L. S. Porfirio Will Castro, R. R. Andreguetti, J. F. Gomes Correia, D. W. Filho, C. T. Pich, R. C. Pedrosa (2016). *Piper nigrum* ethanolic extract rich in piperamides causes ROS overproduction, oxidative damage in DNA leading to cell cycle arrest and apoptosis in cancer cells. Journal of Ethnopharmacology, 16: 30291-30294

Ewton D. Z., J. Hu, M. Vilenchik, X. Deng, K. C. Luk, A. Polonskaia, A. F. Hoffman, K. Zipf, J. F. Boylan, E. A. Friedman (2011). Inactivation of mirk/dyrk1b kinase targets quiescent pancreatic cancer cells. Molecular Cancer Therapeutics, 10: 2104-2114.

Guerrero R. F., B. Puertas, M. I. Fernández, M. Palma, E. Cantos-Villar (2010). Induction of stilbenes in grapes by UV-C: Comparison of different subspecies of Vitis. Innova. Food Sci. Emerg., 11: 231-238.

Han B. J., W. Li, G. B. Jiang, S. H. Lai, C. Zhang, C. C. Zeng, Y. J. Liu (2015). Effects of daidzein in regards to cytotoxicity in vitro, apoptosis, reactive oxygen species level, cell cycle arrest and the expression of caspase and Bcl-2 family proteins. Oncology Reports, 34: 1115-1120.

Han X., S. Deng, N. Wang, Y. Liu, X. Yang (2016). Inhibitory effects and molecular mechanisms of tetrahydrocurcumin against human breast cancer MCF-7 cells. Food & Nutrition Research, 60: 30616, 11 pages.

Hijazi A., H. Bandar, H. Rammal, A. Hachem, Z. Saad, B. Badran (2013). Techniques for the extraction of bioactive compounds from Lebanese *Urtica dioica*. American Journal of Phytomedicine and Clinical Therapeutics, 1(6): 507-513.

Jantova S., N. Mrvova, R. Hudec, J. Sedlak, M. Panik, V. Milata (2016). Pro-apoptotic effect of new quinolone 7- ethyl 9-ethyl-6-oxo-6,9-dihydro[1,2,5]selenadiazolo [3,4-h]quinoline-7-carboxylate on cervical cancer cell line HeLa alone/with UVA irradiation. Toxicology in vitro: An International Journal Published in Association with BIBRA, 33: 35-44.

Kiassos E., S. Mylonaki, D. P. Makris, P. Kefalas (2009). Implementation of response surface methodology to optimise extraction of onion (*Allium cepa*) solid waste phenolics. Innov. *Food Sci. Emerg. Technol.*, 10: 246-252.

Kim E. H., C. X. Deng, M. B. Sporn, K. T. Liby (2011). CDDO-imidazolide induces DNA damage, G2/M arrest and apoptosis in BRCA1-mutated breast cancer cells. Cancer Prev. Res. (Philadelphia, Pa), 4: 425-434.

Kita Y., Y. Miura, K. Yagasaki (2012). Antiproliferative and anti-invasive effect of piceatannol, a polyphenol present in grapes and wine, against hepatoma AH109A cells. J. Biomed. Biotechnol., pp. 1-7.

Kitanovic A., T. Walther, M. O. Loret, J. Holzwarth, I. Kitanovic, F. Bonowski, N. Van Bui, J. M. Francois, S. Wolfl (2009). Metabolic response to MMS-mediated DNA damage in *Saccharomyces cerevisiae* is dependent on the glucose concentration in the medium. FEMS Yeast Research, 9(4): 535-551.

Kidøy L., A. M. Nygård, Ø. M. Andersen, A. T. Pedersen, D. W. Aksnes, B. T. Kiremire (1997). Anthocyanins in fruits of *Passiflora edulis* and *P. suberosa*. J. Food Compos. Anal., 10(1): 49-54.

Kumar C. G., Y. Poornachandra, C. Chandrasekhar (2015). Green synthesis of bacterial mediated anti-proliferative gold nanoparticles: inducing mitotic arrest (G2/M phase) and apoptosis (intrinsic pathway). Nanoscale, 7: 18738-18750.

Kwan Y. P., T. Saito, D. Ibrahim, F. M. Al-Hassan, C. Ein Oon, Y. Chen, S. L. Jothy, J. R. Kanwar, S. Sasidharan (2016). Evaluation of the cytotoxicity, cell-cycle arrest, and apoptotic induction by *Euphorbia hirta* in MCF-7 breast cancer cells. Pharmaceutical Biology, 54: 1223-1236.

Kwon J. Y., S. G. Seo, Y. S. Heo, S. Yue, J. X. Cheng, K. W. Lee, K. H. Kim (2012). Piceatannol, a natural polyphenolic stilbene, inhibits adipogenesis via modulation of mitotic clonal expansion and insulin receptor-dependent insulin signaling in the early phase of differentiation. J. Biol. Chem., 287(14): 11566-11578.

Lai T. N. H., C. André, R. Chirinos, T. B. T. Nguyen, Y. Larondelle, H. Rogez (2014). Optimisation of extraction of piceatannol from *Rhodomyrtus tomentosa* seeds using response surface methodology. Sep. Purif. Technol., 134: 139-146.

Li T., J. Zhu, L. Guo, X. Shi, Y. Liu, X. Yang (2013). Differential effects of polyphenols-enriched extracts from hawthorn fruit peels and fleshes on cell cycle and apoptosis in human MCF-7 breast carcinoma cells. Food Chemistry, 141: 1008-1018.

Matsui Y., K. Sugiyama, M. Kamei, T. Takahashi, T. Suzuki, Y. Katagata, T. Ito (2010). Extract of passion fruit (*Passiflora edulis*) seed containing high amounts of piceatannol inhibits melanogenesis and promotes collagen synthesis. J. Agric. Food Chem., 58: 11112-11118.

Matsui Y., M. Kamei, K. Sugiyama, Piceatannol-containing composition and method of producing piceatannol-containing composition, Google Patents, URL http://www.google.com/patents/US20120004322 (2012). Last accessed on 03 March 2014.

Morton J. (1987). Passion fruit. *In:* Fruits of warm climates. Morton J.F., Miami, FL. pp. 320-328.

Ovesná Z., K. Kozics, Y. Bader, P. Saiko, N. Handler, T. Erker, T. Szekeres (2006). Antioxidant activity of resveratrol, piceatannol and 3,3',4,4',5,5'-hexahydroxy-trans-stilbene in three leukemia cell lines. Oncol. Rep., 16 (3): 617-624.

Shao X., D. Gao, Y. Wang, F. Jin, Q. Wu, H. Liu (2016). Application of metabolomics to investigate the antitumor mechanism of flavopiridol in MCF-7 breast cancer cells. Journal of Chromatography B, Analytical Technologies in the Biomedical and Life Sciences, 1025: 40-47.

Son P. S., S. A. Park, H. K. Na, D. M. Jue, S. Kim, Y. J. Surh (2010). Piceatannol, a catechol-type polyphenol, inhibits phorbol ester-induced NF-kB activation and cyclooxygenase-2 expression in human breast epithelial cells: cysteine 179 of IKKb as a potential target. Carcinogenesis, 31(8): 1442-1449.

Thong B. S., C. Butiman, K. Jitsaeng (2014). Optimised ultrasonic-assisted extraction of antioxidant from mulberry (*Morus alba* L.) leaves using multiple linear regression analysis. Int. J. Pharm. Pharm. Sci., 6(2): 914-917.

Vo N. T., S. Madlener, Z. Bago-Horvath, I. Herbacek, N. Stark, M. Gridling, P. Probst, B. Giessrigl, S. Bauer, C. Vonach (2010). Pro-and anticarcinogenic mechanisms of piceatannol are activated dose dependently in MCF-7 breast cancer cells. Carcinogenesis, 31(12): 2074-2081.

Wang J., Y. M. Zhao, Y. T. Tian, C. L. Yan, C. Y. Guo (2013). Ultrasound-assisted extraction of total phenolic compounds from *Inulahelenium*. Hindawi - The Scientific World Journal, pp. 1-5.

Zhou C., X. Li, C. D. Sun, K. S. Chen (2011). Ultrasonic extraction of flavonoids and phenolics from loquat (*Eriobotrya japonica* Lindl.) flowers. Afr. J. Biotechnol., 10(25): 5020-5026.

Zeng C. C., S. H. Lai, J. H. Yao, C. Zhang, H. Yin, W. Li, B. J. Han, Y. J. Liu (2016). The induction of apoptosis in HepG-2 cells by ruthenium (II) complexes through an intrinsic ROS-mediated mitochondrial dysfunction pathway. European Journal of Medicinal Chemistry, 122: 118-126.