Research Journal of Pharmacognosy (RJP) 8(4), 2021: 79–90
Received: 17 July 2021
Accepted: 10 Sep 2021
Published online: 14 Sep 2021
DOI: 10.22127/RJP.2021.295529.1746

Original article

Cytotoxic Properties, Anthocyanin and Furanocoumarin Content of Red-Pigments Obtained from *Callistemon citrinus* (Curtis) Skeels Flowers

Ardalan Pasdaran[1], Negar Azarpira[2], Nastaran Yaghoobi Solut[3], Maryam Zare[3], Azar Hamedi[4], Nazila Karami[3], Azadeh Hamedi[5*]

[1]Medicinal Plants Processing Research Center, Shiraz University of Medical Sciences, Shiraz, Iran.
[2]Transplant Research Center, Shiraz University of Medical Sciences, Shiraz, Iran.
[3]Student Research Committee, School of Pharmacy, Shiraz University of Medical Sciences, Shiraz, Iran.
[4] Department of Plant Protection, School of Agriculture, Shiraz University, Shiraz, Iran.
[5]Department of Pharmacognosy, School of Pharmacy, Shiraz University of Medical Sciences, Shiraz, Iran.

Abstract

Background and objectives: There is growing interest in introducing safe and bioactive natural red pigments to the pharmaceutical and cosmetic industries. This study was designed to determine the phytochemical content and potential cytotoxicity of red pigment from *Callistemon citrinus* (Curtis) Skeels (syn. *Melaleuca citrina* (Curtis) Dum.Cours.) flowers. **Methods**: The flowers' anthocyanin rich pigment was extracted with ethanol (70%, v/v) containing 0.5% formic acid. This extract was fractionated by a three-step process through a Sep-Pak C_{18} cartridge with water, ethyl acetate, and methanol, respectively. The anthocyanin and coumarin content of the red pigment was identified based on a reverse phase high performance liquid chromatography-electrospray ionization mass spectrometry (HPLC/ESI-MS/MS). The MTT assay was used to assess the cytotoxicity of red pigment evaluated on normal human foreskin fibroblasts as well as two malignant cell lines: human breast cancer (MCF-7) and human fibrosarcoma cells (HT1080) at 24, 48 and 72 hours. **Results**: The anthocyanin compounds in the red pigment fraction were cyanidin (1), cyanidin 3,5-*O*-diglucoside (2), cyanidin 3-*O*-glucoside (3), and cyanidin 3-*O*-glucoside-8-ethyl-catechin (4). Flowers' pigment also contained two furanocoumarins, including 8-(but-2-en-2-yl)-8,9-dihydro-2H-furo[2,3-h] chromen-2-one (or 15-methyl angenomallin, (5)) and 9-methyl-7H-furo[3,2-g] chromen-7-one (or 8-methylpsoralen, (6)). According to the MTT assays, the highest cytotoxic effect was observed on human foreskin fibroblasts with an IC_{50} values of <12.5 (µg/ mL, 24 h) and 85.2 (µg/ mL, 48 h). **Conclusion**: It might be assumed that application of the red pigment of *C. citrinus* in topical formulations and cosmetics should be done with caution due to the observed cytotoxicity on dermal fibroblasts.

Keywords: anthocyanin; biological pigments; cytotoxicity; fibroblasts; heterocyclic compounds

Citation: Pasdaran A, Azarpira N, Yaghoobi Solut N, Zare M, Hamedi A, Karami N, Hamedi A. Cytotoxic properties, anthocyanin and furanocoumarin content of red pigments obtained from *Callistemon citrinus* (Curtis) Skeels flowers. Res J Pharmacogn. 2021; 8(4): 79–90.

Introduction

There is growing interest in natural red pigments from plants in the food and cosmeceutical industries. In general, among different classes of phytochemicals, carotenoids (mainly lycopene), anthocyanins, and betacyanins produce red colors in plant-based pigments. Red-pigments containing a high amount of anthocyanin are known to possess anti-oxidant, anti-inflammatory and anti-angiogenic properties [1]. The aim of this study was to determine the phytochemical

*Corresponding author: hamediaz@sums.ac.ir

components of the red pigment of the cultivated plant *Callistemon citrinus* (Curtis) Skeels (syn. *Melaleuca citrina* (Curtis) Dum.Cours.) (Myrtaceae), which is commonly known as the common red bottlebrush, crimson bottlebrush, or lemon bottlebrush. The plant is native to Australia, but is also cultivated widely in other countries as an ornamental tree due to its beautiful red flowers.

The *Callistemon* genus is a member of the Myrtaceae family. The plants in this genus grow mainly as small shrubs or medium-sized trees. Previous investigations have shown that this genus is a rich source for a variety of compounds, including hydrolysable/non-hydrolysable tannins, phloroglucinols, flavonoids/flavonoid glycosides, triterpenoids, and essential oils [2-5]. *Callistemon* (*Melaleuca*) species have been introduced as good sources of various phloroglucinols. For example, gallomyrtucommulone A, E, F, myrtucommulone B, callistenone B, and endoperoxide G3 have been isolated from *M. citrina* leaves and stems. As other examples, viminalins H, L, N and callisretones A, B with anti-inflammatory effects were isolated from *M. linearis* Schrad. & J.C.Wendl. (synonym of *C. rigidus* R.Br. Stiff bottlebrush) [6,7]. Several studies have also reported the presence of flavanone compounds such as leucadenone A-D (four β-triketone flavanones), which have been isolated from *M. leucadendron* L.[5,8]. Some isolated flavonoids from *M. lanceolatus* DC. including 4 ,5-dihydroxy-6,8-dimethyl-7-methoxyflavanone, eucalyptin, 8-demethyleucalyptin, sideroxylin, syzalterin, and quercetin have shown potential efficacy against Alzheimer's disease [9].

Among other classes of phenolic compounds, chromones are an important group of *Melaleuca* genus compositions. For example, melachromone, 5-hydroxy-7-methoxy-2,6,8-trimethylchromone, 2,5-dihydroxy-7-methoxy-2, 6-dimethylchromanone, eugenitin, and noreugenin are the chromones isolated from *M. cajuputi* Powell leaves [10].

Although some investigations have been reported on the pigment compositions of *Callistemon* species (mostly hydrolysable tannins), just a few reports can be found on the anthocyanin or coumarin content of the pigments obtained from this genus. This study investigated the anthocyanins and furanocoumarin content of the red-pigment obtained from flowers of *C. citrinus* using the HPLC/ESI-MS/MS method. Also, the potential toxicity of this pigment was determined against normal human foreskin fibroblasts as well as cancerous cells using the MTT assay.

Materials and Methods

Ethical considerations

This study was an in vitro study and did not involve any humans or animals. The authors considered ethics in research in the study. The study was approved by ethical committee of Shiraz University of Medical Sciences (IR.SUMS.REC.1400.396).

Plant materials

Callistemon citrinus flowers were collected from cultivated trees in the Shiraz urban region, Fars province (29°34'48.1"N 52°36'36.1"E, with an altitude of 1499m) during the flowering season (10^{th} of May 2019). This species was identified by Dr. Ardalan Pasdaran (pharmacognosist) and authorized under voucher specimen number (MPPRC-00-01) at the Herbarium of Medicinal Plants Processing Research Center, Shiraz University of Medical Sciences, Shiraz, Iran.

Chemicals

Human breast cancer (MCF-7) and human fibrosarcoma cells (HT1080) were purchased from the Pasteur Institute (Tehran, Iran). The human foreskin fibroblasts were obtained from the Transplant Research Center (Shiraz, Iran). Solvents were of high purity and purchased from Dr. Mojallali Industrial Chemical Complex Co. (Tehran, Iran). All other chemicals were purchased from Sigma-Aldrich (USA) or Merck (Merck Millipore, Germany). The Sep-Pak C18 cartridge was purchased from Waters Assoc. (Milford, USA). Trypsin was purchased from Gibco (Italy).

Anthocyanin extraction and fractionation

Air dried *C. citrinus* flowers were subjected to extraction. The flower powder (5 kg) was immersed in 10 liters of ethanol (70%, v/v) containing 0.5% formic acid. Previous works have shown that a higher percentage of ethanol would decrease extraction of polysaccharides and increase the extraction rate of phenolic compounds, including anthocyanins. After three times of extraction (30 min for each step), the combined supernatants were concentrated and dried with a rotary evaporator at 30 °C. The dried extract (10 g) was fractionated on a Sep-Pak C_{18} cartridge Simple organic acids, sugars, and other

highly water-soluble compositions were washed away with 300 mL of an aqueous solution containing 0.01% hydrochloric acid (v/v, fraction A). According to the Oszmianski and Lee method [11], other phenolics such as flavonoids and non-anthocyanin compounds were separated from anthocyanins by using ethyl acetate as an intermediate mobile phase (300 mL, fraction B). In the final step, the anthocyanins fraction (fraction C) was subsequently eluted by 300 mL of methanol containing 0.01% hydrochloric acid (v/v). This final fraction was concentrated and used as the anthocyanin rich fraction for further analysis [11,12].

HPLC/ESI-MS/MS analysis

Anthocyanin rich fraction (fraction C) was eluted on a C_{18} column (Atlantis T3-C18 3µ, 2.1×100 mm) using a Waters Alliance 2695 HPLC-Micromass Quattro micro–API Mass Spectrometer system. The following conditions were used for the HPLC procedure: acetonitrile and water (each containing 0.1% formic acid) with a linear gradient (5-95%) from 0 to 25 min; and an isocratic gradient (95%) from 25 to 35 min. at a flow rate of 0.25 mL/min were used as the mobile phase and the column temperature was 35 °C.

The mass data was obtained on a Waters Quattro micro-API Mass triple quadrupole ion mass spectrometer coupled with HPLC. The quadrupole equipment was set at the following conditions: Mode, ESI^+; capillary voltage, 4.5 kV; cone voltage, 35V; RF lense, 0.2V; extractor, 2 V; desolvation gas temperature, 300 ^oC at a flow of 200 L/h (N_2, grade 5); source temperature, 120 ^oC; and collision energy, 45 eV.

Assessment of cell proliferation based on MTT assay

The human foreskin fibroblasts, human breast cancer (MCF-7), and human fibrosarcoma cells (HT1080) were purchased from the Pasteur Institute of Iran. Human foreskin fibroblasts were cultured in DMEM/F12 (Dulbecco's modified Eagle's medium (DMEM), DMEM/Ham's nutrient mixture F12). MCF-7 and HT1080 cells were cultured in RPMI1640 medium. All media were supplemented with 10% fetal bovine serum (FBS), and penicillin G (100 IU/mL). The cultures were incubated at 37 °C in a humidified atmosphere containing 95% air/5% CO_2 in a 75-cm2 sterile flask. To obtain the cells, they were trypsinized (1 mmol) in the late log-phase of growth, centrifuged (1500 g for 5 min), and re-suspended in the culture medium [6]. The cells were counted and seeded into 96-well plates at a density of 7000 cells per well.

The cytotoxicity of the anthocyanin rich fraction at different concentrations (12.5, 25, 50, 100, and 200 µg/mL) on the cultured cells was detected using the 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) colorimetric method. Paclitaxel was used as the positive control with similar concentrations. Blank wells and wells with cells which received only media were considered as untreated controls. After 24, 48, and 72 h of incubation, 100 µL of each well medium was discarded and 100 µL of MTT solution (0.5 mg/ mL in phosphate-buffered saline (PBS), pH= 7.2) was added into each well. After 4 h of exposure and centrifugation at 3000 rpm, the supernatants were discarded and 100 µL of dimethyl sulfoxide (DMSO) was added to each well. The absorbance of blank, controls and treated wells was measured using an enzyme-linked immunosorbent assay (ELISA) plate reader BioTek Instruments®, USA at 570 and 630 nm [6].

Statistical analysis

The mean and standard deviation (SD) of at least three experiments were used to represent all data. The IC_{50} values in the cytotoxicity tests was calculated using GraphPad Prism version 7 (GraphPad Prism Software, USA). Furthermore, Tukey's multiple comparison test and two-way analysis of variance (ANOVA) were employed to statistically compare the groups. The significant level was considered $p \leq 0.05$.

Results and Discussion

The chemical structures of compounds were established based on direct LC/MS/MS data analysis and comparison with previous literatures [13,14].

According to the LC/MS/MS spectrum, cyanidin (compound 1, Figure 1a) was detected in MS^1 with *m/z* 288 and four daughter ions at *m/z* 153, 121, and 213 in MS^2. Some investigations on the Myrtaceae genus, including *Eugenia*, *Metrosideros*, and *Syzygium* have shown that 3,5-diglucosides anthocyanins such as cyanidin, delphinidin, petunidin and malvidin present as characteristic anthocyanin diglucosides in these genus [15,16]. In an investigation on the

anthocyanins content of native Australian fruits using the HPLC-DAD/ESI/MS-MS method, Netzel et al. reported the presence of cyanidin 3,5-diglucoside with *m/z* 611, 449, and 287 as typical fragments in *Syzygium paniculatum* Gaertn [17]. Similar reports also exist about *Eugenia myrtifolia* Sims., *Syzygium cumini* (L.) Skeels., and *Syzygium malaccense* (L.) Merr. & LM Perry [18-20]. According to these literatures, compound 2 (Figure 1b) with a *m/z* of 611 as the parent ion in MS^1, daughter ions with *m/z* 449, and 287 (MS^2) corresponded to cyanidin 3,5-*O*-diglucoside. The daughter ion resulted from losing glucose fragments which were presented at 3 and 5- substitution of the anthocyanin.

The other observed parent ion at *m/z* 450 (MS^1, Figure 2a) with the MS^2 fragment at *m/z* 287 is correlated to 3 substituted cyanidin. Therefore, the structure of compound 3 was suggested as cyanidin 3-*O*-glucoside. Several reports can be found in literature on the identification of cyanidin 3-*O*-glucoside with a similar MS-MS pattern in the plants of the Myrtaceae family, such as *Eugenia spp.*, *Syzygium spp.*, and *Myrcianthes spp.* [21-23].

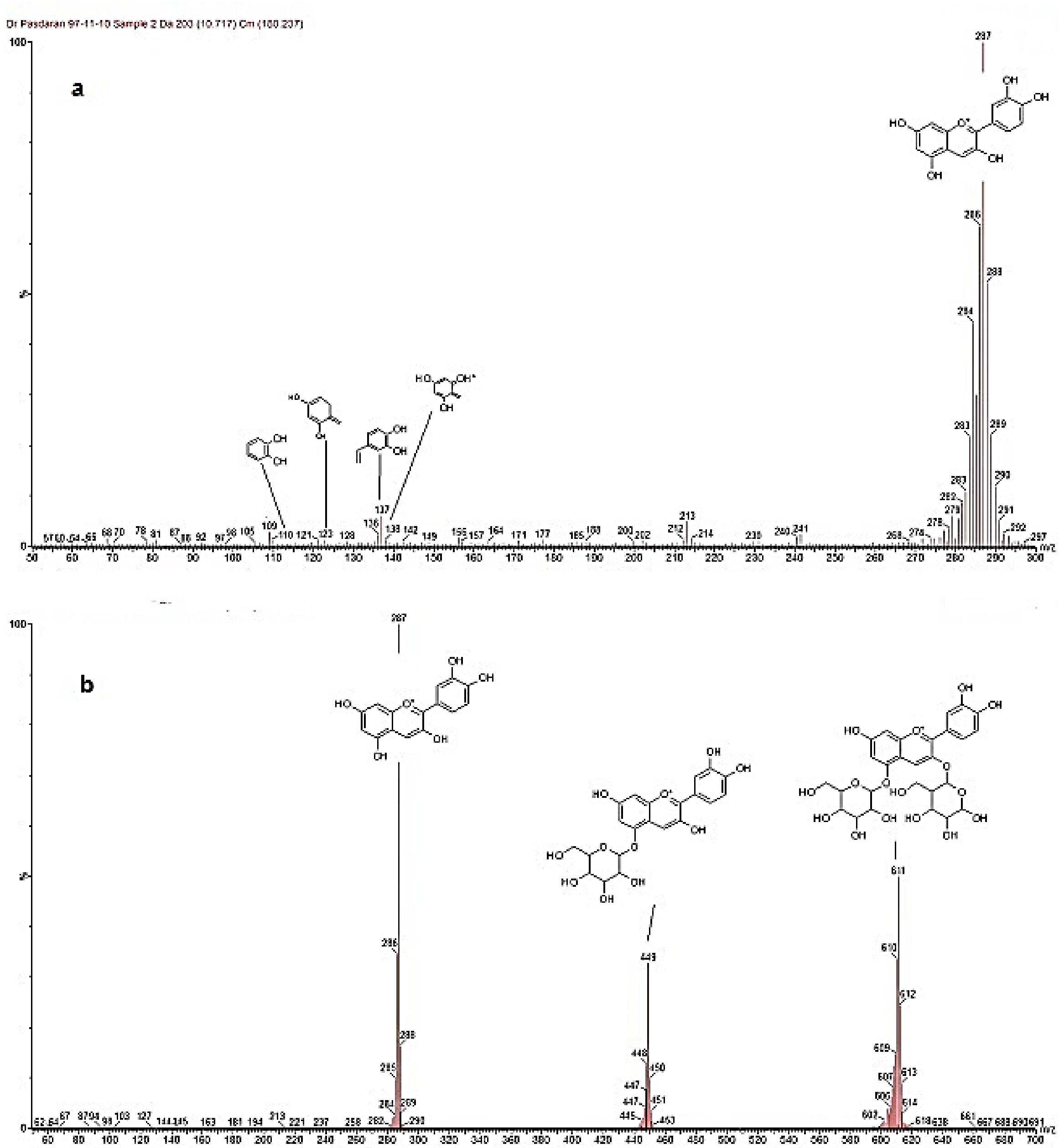

Figure 1. Positive ion ESI-MS spectrum and fragments of cyanidin (a) and cyanidin 3,5-*O*-diglucoside (b)

The hydrogen $[M+H]^+$ adduct ion was observed with a good abundance at *m/z* 766 (Figure 2b). Loss of one unit of glucose from the C-O glycosidic bound of compound 4 produces a low abundance ion at *m/z* 605 $[M-162]^+$ (fragment A). The next fragmented ion at *m/z* 453 resulted from the loss of the 2-(3,4-dihydroxyphenyl) acetaldehyde moiety from fragment A. Further fragmentation resulted in a cyanidin moiety with *m/z* 287. According to previous reports, the ion at *m/z* 315 (a high relative abundance signal product ion) corresponds to a vinyl-catechin adduct [24]. Another observed ion product at *m/z* 453, which is a common observed ion in procyanidins dimers, corresponded to Retro-Diels-Alder fragmentation in this category of pigments [25]. Therefore, based on mass data and compression with previous works, compound 4 was determined as 3-*O*-glucoside-8-ethyl-catechin [24].

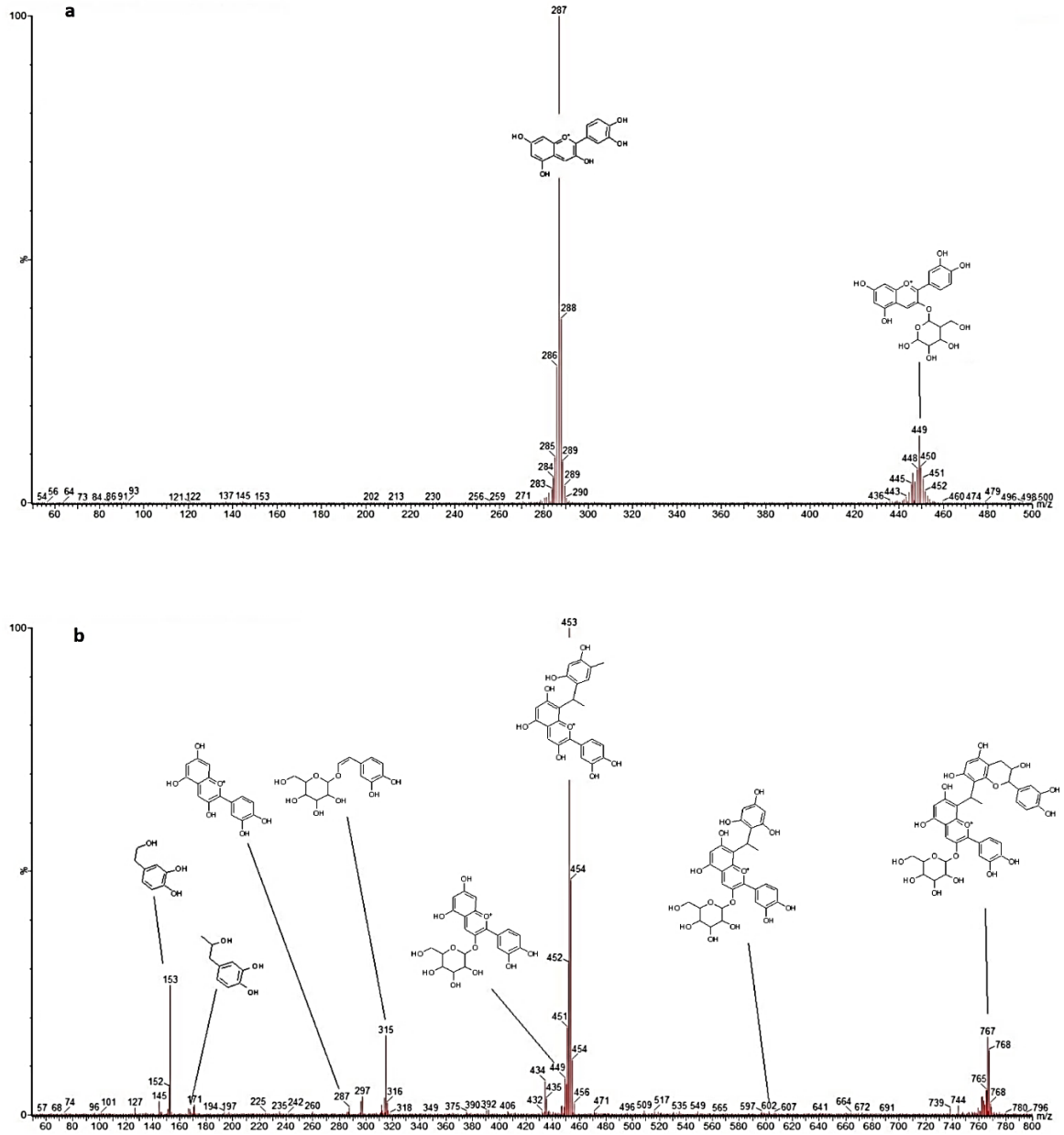

Figure 2. Positive ion ESI-MS spectrum and fragments of cyanidin 3-*O*-glucoside (a) and cyanidin 3-*O*-glucoside-8-ethyl-catechin (b)

Although anthocyanins with an ethyl-bridged catechin backbone are an important natural pigment in wine, they have been reported from other plant sources, for example from *Vaccinium macrocarpon* Aiton. Stevens variety, and *Vaccinium vitis-idaea* L. [26,27]. In addition to the described anthocyanins (Table 1) in this study, two furanocoumarins were also determined from *C. citrinus* flowers. The identified furanocoumarins are 8-(but-2-en-2-yl)-8,9-dihydro-2H-furo[2,3-h] chromen-2-one (or 15-methyl angenomallin, compound 5, Figure 3a) and 9-methyl-7H-furo [3,2-g] chromen-7-one (known as 8-methylpsoralen, compound 6. Figure 3b).

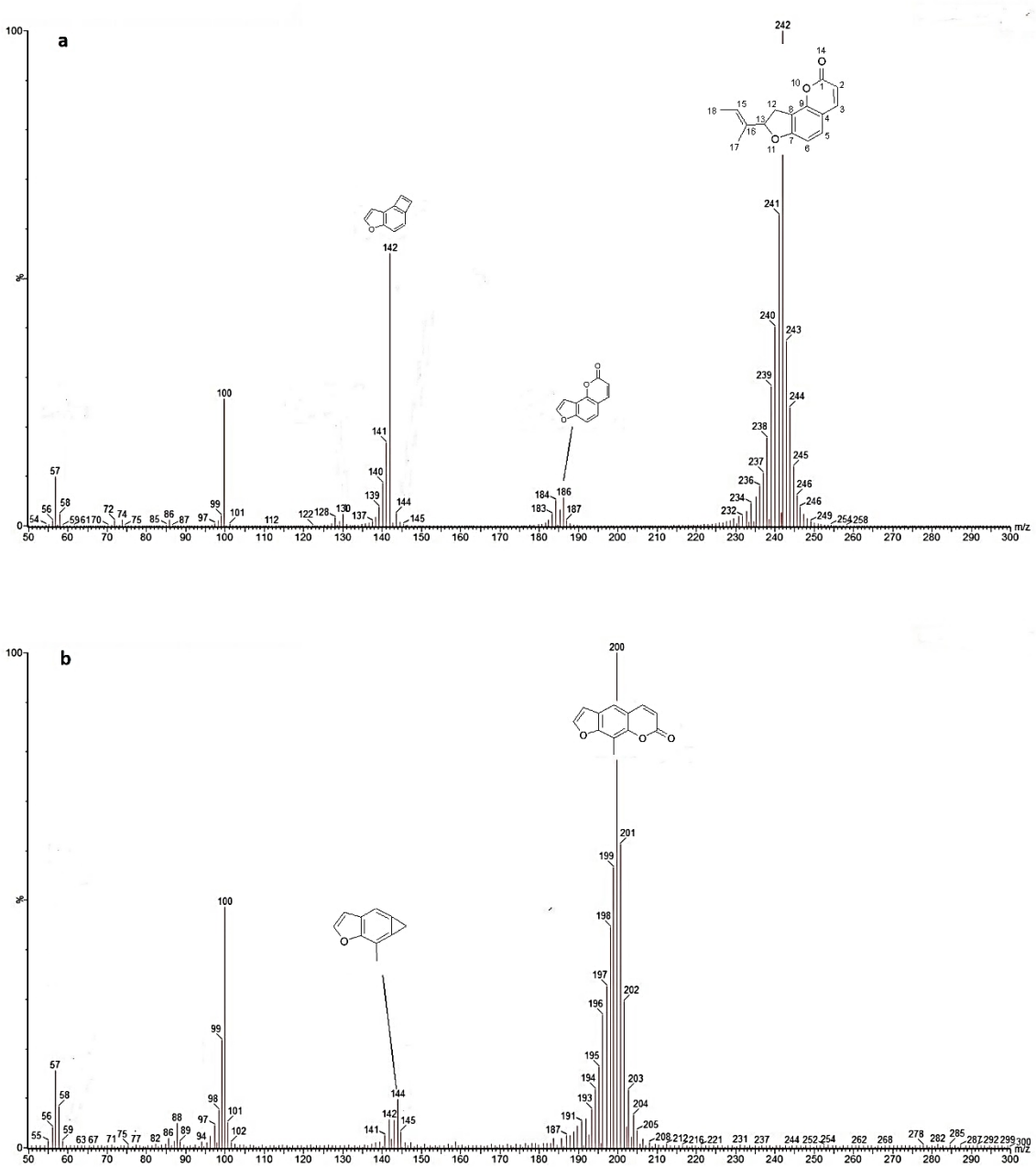

Figure 3. Positive ion ESI-MS spectrum and fragments of 8-(but-2-en-2-yl)-8,9-dihydro-2H-furo[2,3-h] chromen-2-one (a) and 9-methyl-7H-furo[3,2-g] chromen-7-one (b)

Table 1. Anthocyanins and furanocoumarins in red-pigment of *Callistemon citrinus* (Curtis) Skeels flowers identified by HPLC/ESI-MS/MS (positive mode) and their principal mass fragments

	Anthocyanin		
	Compounds	**MS^1 (m/z)**	**MS^2 (m/z)**
1	Cyanidin	288	153, 121, 213
2	Cyanidin 3,5-*O*-diglucoside	611	449, 287
3	Cyanidin 3-*O*-glucoside	450	287
4	Cyanidin 3-*O*-glucoside-8-ethyl-catechin	766	453, 315, 153
	Furanocoumarin		
5	8-(but-2-en-2-yl)-8,9-dihydro-2H-furo[2,3-h] chromen-2-one (or 15-methyl angenomallin)	242	186, 142, 100, 57
6	9-methyl-7H-furo[3,2-g] chromen-7-one (or 8-methylpsoralen).	200	144, 100, 57

MS^1: parent ion in mass spectrum; MS^2: daughter ion in mass spectrum

As seen in Figure 4, different concentrations of the red pigment of *C. citrinus* had a range of cytotoxicity on all examined cell lines.

The highest cytotoxicity was observed on human foreskin fibroblasts, and even at the lowest examined concentration (12.5 µg/mL), the proliferation of the fibroblast cells was inhibited by up to 67% thus the IC_{50} value on human foreskin fibroblasts was estimated< 12.5 (µg/ mL) at 24-h time point. This effect was decreased at 48 h time pint ($p \leq 0.05$) with the IC_{50} value of 85.2 (µg/ mL). However, the number of living cells was increased after 72 h of exposure to the lower concentrations of the pigment, which shows a proliferative pattern.

The extracted pigment did not exhibit considerable cytotoxicity on MCF-7 and HT1080 cells at low concentrations. But the inhibition of cell proliferation reached 60% at high concentrations (100-200 µg/mL). After 72 h of exposure, the MCF-7 and HT1080 cells showed a proliferative pattern at low concentrations (12.5-25 µg/mL). It is likely that the molecules added to the culture media were consumed or metabolized by the cells during the first hours (especially at lower concentrations). Because the cytotoxicity was not 100%, the remaining surviving cells began to grow during the remaining time (to 72 hours) to compensate for the loss, which might explain the proliferative pattern at the 72 h time point.

One recent study on the anthocyanin content of *C. citrinus* flowers reported the presence of cyanidin 3,5-*O*-diglucoside and cyanidin 3-*O*-glucoside, which is in accordance with the results of the present study [28]. However, in contrast to this report, we could not detect peonidin-3,5-*O*-diglucoside and cyanidin-coumaroyl glucoside pyruvic acid. Also, we have detected two other anthocyanins, including cyanidin and cyanidin 3-*O*-glucoside-8-ethyl-catechin. Moreover, we detected two furanocoumarins including 8-(but-2-en-2-yl)-8,9-dihydro-2H-furo[2,3-h] chromen-2-one (or 15-methyl angenomallin) and 9-methyl-7H-furo[3,2-g] chromen-7-one (which is also known as 8-methylpsoralen). The difference between the present study and the Laganà et al. report may arise from the differences in the applied method for anthocyanin extraction as well as the instruments which were used for structure elucidation. Also, the ecological condition of where the plants grow has a significant impact on their anthocyanin contents [28].

Mass spectrometry is one of the common methods to identify natural compound [29]. Several reports have applied LC-MS spectrometry for structure elucidation of furanocoumarins. For example, Yu at al., have identified nine new furanocoumarins from grapefruit juice retentate [13]. Jain et al., have reported two new furanocoumarins, including 5-(1" ,1" -dimethylallyl)-8-methyl psoralen and d 2" -O-acetyl oxypeucedanin hydrate-3" -methyl ether from *Ficus carica* root heartwood [14]. Heinke et al., applied liquid chromatography/electrospray tandem mass spectrometry to analyze a series of prenylated furanocoumarins from the leaves of Yemenite Dorstenia species. Several derivatives of psoralen (ficusin) have been identified in these plants [30]. We have detected 8-methyl psoralen; this compound and its derivatives have been identified in several plants, including hogweed, *Ficus carica* and some other plants in Umbelifercea and Rutaceae [14,30-32]. The furanocoumarin angenomallin was also reported from some plant species, such as *Notopterygium incisum* [33] and *Angelica anomala* [34].

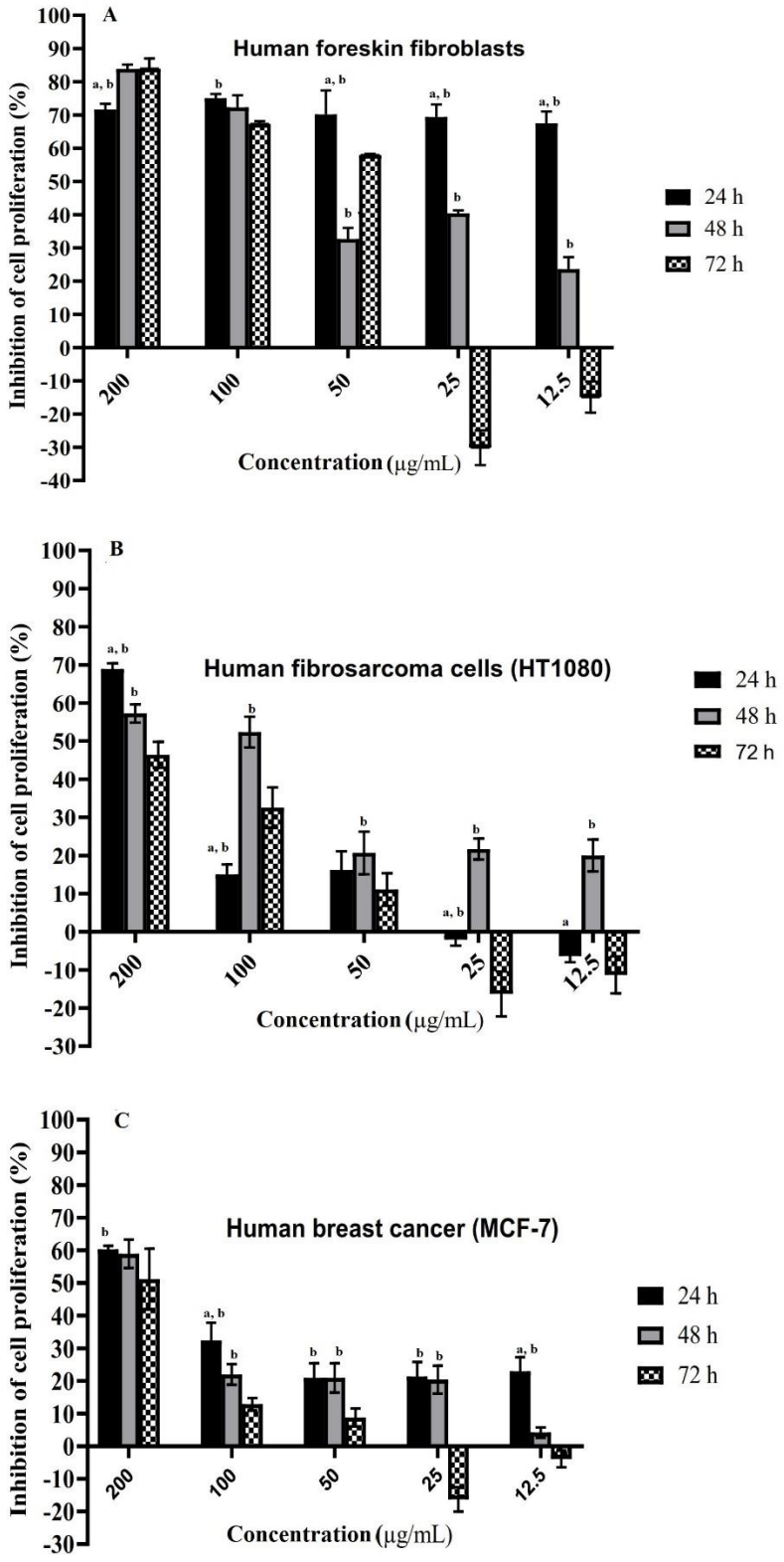

Figure 4. Inhibition of cell proliferation by different concentrations of red pigment obtained from *Callistemon citrinus* flowers; A) normal human foreskin fibroblasts; B) HT1080 (human fibrosarcoma cells); C) MCF-7 (human breast cancer) using MTT assay at 24, 48 and 72 hours; the data is represented as Mean ± SD and each experiment was done three times, independently.

Very limited reports on the presence of some simple and complex coumarins in the *Myrtaceae* family can be found in literature. Esculin from *Myrtus communis* L. and three phenylcoumarins (Costatamins A–C) with anti-inflammatory activity from *Angophora costata* (Gaertn.) Britten are some examples of the coumarins in Myrtaceae plants [35,36].

The acidified methanolic extract of *C. citrinus* flowers has been recently reported to have antioxidant, cytoprotective, and anti-angiogenic properties. This extract was reported to have no significant changes in the vitality of mononuclear cells (0.0–100.0 µg/mL) while at higher concentrations (125, 150, 175, 200 µg/mL), the cell mortality reached 50% [28]. In our study, we observed a high cytotoxicity for *C. citrinus* red pigment on human foreskin fibroblast cell line even at low concentrations. It also had a range of cytotoxicity on MCF-7 and fibrosarcoma cell lines at different concentrations. The differences in the results of these reports might be due to the different cell lines as well as the differences in the phytochemical constituents of the extracted pigments applied in these studies. It is unclear if the observed cytotoxicity was induced by the anthocyanins or the furanocoumarins content of the red pigment fraction of *C. citrinus*. In particular, several furanocoumarins are well known for their cytotoxicity on dermal cells [37-39]. Nevertheless, previous reports showed that cyanidin and its glycosides (the main anthocyanin components of the red-pigment obtained from *C. citrinus*) could exhibit cytotoxic effects on different cell lines, such as human monocytic leukemia cells [40], glioblastoma cell lines [41], and prostate cancer cells [41,42]. Polyphenols such flavonoids and anthocyanins have shown a variety of biological activities in clinical trials [43]. Because of their antioxidant characteristics, anthocyanins have attracted special interest as cosmetic and therapeutic phytochemicals for the skin [44]. Although anthocyanins are easily absorbed and detected in plasma after oral consumption, they have low bioavailability after ingestion. Therefore, they are generally detected in very low concentrations in human plasma after absorption. It is believed that only 1% of the total anthocyanin intake will be detected [1,45]. Anthocyanin penetration into the stratum corneum, which is required for bioactivity, is poorly understood. However, previous studies have shown that some anthocyanins are able to penetrate the skin and reach depths [46,47].

Conclusion

In the present study, the red pigment of *C. citrinus* showed cytotoxic effects on human foreskin fibroblasts even at low concentrations. Dermal fibroblasts are cells that reside in the dermis layer of the skin and are responsible for the creation of collagen, an extracellular matrix. They also play an important role in wound healing. In animals, fibroblasts are the most frequent connective tissue cells. They are crucial in the immunological response to tissue damage.

Despite the previous report on the anti-oxidant properties of anthocyanins of *C. citrinus* [28], application of the red pigment of *C. citrinus* in topical anti-oxidant formulations and cosmetics is not recommended due to the observed cytotoxicity on dermal fibroblasts in the present study.

On the other hand, some skin conditions, such as keloids and hypertrophic scars [48], Elejalde syndrome [49], cutaneous mucinoses, scleroderma, scleromyxedema [50,51], and psoriasis are linked to increased proliferation of skin cells such as fibroblasts. Theoretically, using cytotoxic agents on fibroblasts could be beneficial in such disorders.

Acknowledgments

This study was part of the Pharm. D thesis project. The project was funded by Shiraz University of Medical Sciences (Grant No. 22319).

Author contributions

Ardalan Pasdaran, Negar Azarpira, Nastaran Yaghoobi Solut, Maryam Zare, Azar Hamedi, Nazila Karami and Azadeh Hamedi were involved in performing the experiments, analyzing data and preparing the manuscript. Ardalan Pasdaran and Azadeh Hamedi designed the study, interpreted the data and revised the final manuscript.

Declaration of interest

The authors declare that there is no conflict of interest. The authors alone are responsible for the accuracy and integrity of the paper content.

References

[1] Leong HY, Show PL, Lim MH, Ooi CW, Ling TC. Natural red pigments from plants and their health benefits: a review. *Food Rev Int*. 2018; 34(5): 463–482.

[2] Abdelhady MI, Kamal AM, Tawfik NF, Abdelkhalik SM. Polyphenolic constituents of the methanolic extract of *Callistemon viridiflorous* leaves and its antimicrobial activity. *Pharmacogn Mag*. 2012; 4(31): 47–53.

[3] Nazreen S, Kaur G, Alam MM, Shafi S, Hamid H, Ali M, Alam MS. New flavones with antidiabetic activity from *Callistemon lanceolatus* DC. *Fitoterapia*. 2012; 83(8): 1623–1627.

[4] Jeong W, Hong SS, Kim N, Yang YT, Shin YS, Lee C, Hwang BY, Lee D. Bioactive triterpenoids from *Callistemon lanceolatus*. *Arch Pharm Res*. 2009; 32(6): 845–849.

[5] Bar FMA. Genus *Melaleuca*-a review on the phytochemistry and pharmacological activities of the Non-volatile components. *Rec Nat Prod*. 2021; 15(4): 1–24.

[6] Khanh PN, Duc HV, Huong TT, Son NT, Ha VT, Van DT, Tai BH, Kim JE, Jo AR, Kim YH. Alkylphloroglucinol derivatives and triterpenoids with soluble epoxide hydrolase inhibitory activity from *Callistemon citrinus*. *Fitoterapia*. 2016; 109: 39–44.

[7] Cao JQ, Tian HY, Li MM, Zhang W, Wang Y, Wang L, Ye WC. Rearranged phloroglucinol-monoterpenoid adducts from *Callistemon rigidus*. *J Nat Prod*. 2018; 81(1): 57–62.

[8] Lee CK. Leucadenone AD, the novel class flavanone from the leaves of *Melaleuca leucadendron* L. *Tetrahedron Lett*. 1999; 40(40): 7255–7259.

[9] Park SY, Lim JY, Jeong W, Hong SS, Yang YT, Hwang BY, Lee D. C-methylflavonoids isolated from *Callistemon lanceolatus* protect PC12 cells against Aβ-induced toxicity. *Planta Med*. 2010; 76(9): 863–868.

[10] Rattanaburi S, Mahabusarakam W, Phongpaichit S, Carroll AR. A new chromone from the leaves of *Melaleuca cajuputi* Powell. *Nat Prod Res*. 2013; 27(3): 221–225.

[11] Oszmianski J, Lee C. Isolation and HPLC determination of phenolic compounds in red grapes. *Am J Enol Vitic*. 1990; 41(3): 204–206.

[12] Giusti MM, Rodríguez-Saona LE, Griffin D, Wrolstad RE. Electrospray and tandem mass spectroscopy as tools for anthocyanin characterization. *J Agric Food Chem*. 1999; 47(11): 4657–4664.

[13] Yu J, Buslig BS, Haun C, Cancalon P. New furanocoumarins detected from grapefruit juice retentate. *Nat Prod Res*. 2009; 23(5): 498–506.

[14] Jaina R, Jain SC, Bhagchandani T, Yadav N. New furanocoumarins and other chemical constituents from *Ficus carica* root heartwood. *Z Naturforsch C J Biosci*. 2013; 68(1-2): 3–7.

[15] Andersen OM. Proportions of individual anthocyanins in the genus *Metrosideros*. *Biochem Syst Ecol*. 1988; 16(6): 535–539.

[16] Li L, Zhang Y, Seeram NP. Structure of anthocyanins from *Eugenia jambolana* fruit. *Nat Prod Commun*. 2009; 4(2): 217–219.

[17] Netzel M, Netzel G, Tian Q, Schwartz S, Konczak I. Native Australian fruits, a novel source of antioxidants for food. *Innov Food Sci Emerg Technol*. 2007; 8(3): 339–346.

[18] Faria AF, Marques MC, Mercadante AZ. Identification of bioactive compounds from jambolão (*Syzygium cumini*) and antioxidant capacity evaluation in different pH conditions. *Food Chem*. 2011; 126(4): 1571–1578.

[19] Longo L, Scardino A, Vasapollo G, Blando F. Anthocyanins from *Eugenia myrtifolia* Sims. *Innov Food Sci Emerg Technol*. 2007; 8(3): 329–332.

[20] Batista ÂG, da Silva-Maia JK, Júnior MRM. Bioactive compounds of Red-Jambo fruit (*Syzygium malaccense* (L.) Merr. & LM Perry). In: Murthy HN, Bapat VA, Eds. Bioactive compounds in underutilized fruits and nuts. New York: Springer International Publishing, 2020.

[21] Reynertson KA, Yang H, Jiang B, Basile MJ, Kennelly EJ. Quantitative analysis of antiradical phenolic constituents from fourteen edible Myrtaceae fruits. *Food Chem.* 2008; 109(4): 883–890.

[22] Seraglio SKT, Schulz M, Nehring P, Della Betta F, Valese AC, Daguer H, Gonzaga LV, Fett R, Costa ACO. Nutritional and bioactive potential of Myrtaceae fruits during ripening. *Food Chem*. 2018; 239: 649–656.

[23] Chavez Carvajal P, Coppo E, Di Lorenzo A, Gozzini D, Bracco F, Zanoni G, Nabavi SM,

Marchese A, Arciola CR, Daglia MM. Chemical characterization and in vitro antibacterial activity of *Myrcianthes hallii* (O. Berg) McVaugh (Myrtaceae), a traditional plant growing in Ecuador. *Materials*. 2016; 9(6): 1–14.

[24] Rockenbach II, Jungfer E, Ritter C, Santiago-Schübel B, Thiele B, Fett R, Galensa R. Characterization of flavan-3-ols in seeds of grape pomace by CE, HPLC-DAD-MSn and LC-ESI-FTICR-MS. *Food Res Int*. 2012; 48(2): 848–855.

[25] Saucier C, Guerra C, Pianet I, Laguerre M, Glories Y. (+)-Catechin—acetaldehyde condensation products in relation to wine-ageing. *Phytochemistry*. 1997; 46(2): 229–234.

[26] Tarascou I, Mazauric JP, Meudec E, Souquet JM, Cunningham D, Nojeim S, Cheynier V, Fulcrand H. Characterisation of genuine and derived cranberry proanthocyanidins by LC–ESI-MS. *Food Chem*. 2011; 128(3): 802–810.

[27] Brown E, Gill C, Stewart D, Mc Dougall G. Lingonberries (*Vaccinium vitis-idaea* L) and blueberries (*Vaccinium corymbosum*) contain discrete epicatechin anthocyanin derivatives linked by ethyl bridges. *J Berry Res*. 2016; 6(1): 13–23.

[28] Laganà G, Barreca D, Smeriglio A, Germanò MP, D'Angelo V, Calderaro A, Bellocco E, Trombetta D. Evaluation of anthocyanin profile, antioxidant, cytoprotective, and anti-angiogenic properties of *Callistemon citrinus* flowers. *Plants*. 2020; 9(8): 1–14.

[29] Hamedi A, Moheimani SM, Sakhteman A, Etemadfard H, Moein M. An overview on indications and chemical composition of aromatic waters (hydrosols) as functional beverages in Persian nutrition culture and folk medicine for hyperlipidemia and cardiovascular conditions. *J Evid Based Complement Altern Med*. 2017; 22(4): 544–561.

[30] Heinke R, Franke K, Michels K, Wessjohann L, Ali NAA, Schmidt J. Analysis of furanocoumarins from Yemenite *Dorstenia* species by liquid chromatography/electrospray tandem mass spectrometry. *J Mass Spectrom*. 2012; 47(1): 7–22.

[31] Evans FJ, Schmidt RJ. Plants and plant products that induce contact dermatitis. *Planta Med*. 1980; 38(4): 289–316.

[32] Janusz SC, Schwartz RA. Botanical briefs: phytophotodermatitis is an occupational and recreational dermatosis in the limelight. *Cutis*. 2021; 107(4): 187–189.

[33] Kou G, Zhang Y, Yang X, Rong R. O-methylnotopterol, a new natural product from the roots and rhizomes of *Notopterygium incisum*. *Zhongguo Zhong Yao Za Zhi*. 2010; 35(9): 1134–1136.

[34] Hata K, Kozawa M, Ikeshiro Y. The constitution of anomalin, a new coumarin isolated from the root of *Angelica anomala* Lall. (umbelliferae). *Chem Pharm Bull (Tokyo)*. 1966; 14(1): 94–96.

[35] Martín T, Rubio B, Villaescusa L, Fernández L, Díaz AM. Polyphenolic compounds from pericarps of *Myrtus communis*. *Pharm Biol*. 1999; 37(1): 28–31.

[36] Raju R, Singh A, Bodkin F, Münch G. Costatamins A–C, new 4-phenylcoumarins with anti-inflammatory activity from the Australian woodland tree *Angophora costata* (Myrtaceae). *Fitoterapia*. 2019; 133: 171–174.

[37] Conforti F, Marrelli M, Menichini F, Bonesi M, Statti G, Provenzano E, Menichini F. Natural and synthetic furanocoumarins as treatment for vitiligo and psoriasis. *Curr Drug Ther*. 2009; 4(1): 38–58.

[38] Kostova I. Synthetic and natural coumarins as cytotoxic agents. *Curr Med Chem Anticancer Agents*. 2005; 5(1): 29–46.

[39] Ahmed S, Khan H, Aschner M, Mirzae H, Küpeli Akkol E, Capasso R. Anticancer potential of furanocoumarins: mechanistic and therapeutic aspects. *Int J Mol Sci*. 2020; 21(16): 1–20.

[40] Hyun JW, Chung HS. Cyanidin and malvidin from *Oryza sativa* cv. *heugjinjubyeo* mediate cytotoxicity against human monocytic leukemia cells by arrest of G2/M phase and induction of apoptosis. *J Agric Food Chem*. 2004; 52(8): 2213–2217.

[41] Hosseini MM, Karimi A, Behroozaghdam M, Javidi MA, Ghiasvand S, Bereimipour A, Aryan H, Nassiri F, Jangholi E. Cytotoxic and apoptogenic effects of cyanidin-3-glucoside on the glioblastoma cell line. *World Neurosurg*. 2017; 108: 94–100.

[42] Nanashima N, Horie K, Maeda H, Tomisawa T, Kitajima M, Nakamura T.

Blackcurrant anthocyanins increase the levels of collagen, elastin, and hyaluronic acid in human skin fibroblasts and ovariectomized rats. *Nutrients*. 2018; 10(4): 1–15.

[43] Jafarpour M, Yousefi G, Hamedi A, Shariat A, Salehi A, Heydari M. Effect of a traditional syrup from *Citrus medica* L. fruit juice on migraine headache: a randomized double-blind placebo controlled clinical trial. *J Ethnopharmacol*. 2016; 179: 170–176.

[44] Bracone F, De Curtis A, Di Castelnuovo A, Pilu R, Boccardi M, Cilla S, Macchia G, Deodato F, Costanzo S, Iacoviello L. Skin toxicity following radiotherapy in patients with breast carcinoma: is anthocyanin supplementation beneficial? *Clin Nutr*. 2021; 40(4): 2068–2077.

[45] Fernandes I, Faria A, Calhau C, de Freitas V, Mateus N. Bioavailability of anthocyanins and derivatives. *J Funct Foods*. 2014; 7: 54–66.

[46] Westfall A, Sigurdson GT, Rodriguez-Saona LE, Giusti MM. Ex vivo and in vivo assessment of the penetration of topically applied anthocyanins utilizing ATR-FTIR/PLS regression models and HPLC-PDA-MS. *Antioxidants*. 2020; 9(6): 486–500.

[47] Selamoglu Z. Polyphenolic compounds in human health with pharmacological properties. *J Tradit Med Clin Natur*. 2017; 6(4): 1.

[48] Wu W, Xie F, Zhang Y, Wang X, Xia L, Wu X, Gao Z. A novel regulatory function for miR-217 targetedly suppressing fibronectin expression in keloid fibrogenesis. *Int J Clin Exp Pathol*. 2018; 11(4): 1866–1877.

[49] Šilhánová E, Plevová P, Čuřík R, Kasperčík I, Křepelová A. Elejalde syndrome, a case report. *Am J Med Genet A*. 2006; 140(20): 2223–2226.

[50] Georgakis CDC, Falasca G, Georgakis A, Heymann WR. Scleromyxedema. *Clin Dermatol*. 2006; 24(6): 493–497.

[51] Harris JE, Purcell SM, Griffin TD. Acral persistent papular mucinosis. *J Am Acad Dermatol*. 2004; 51(6): 982–988.

Abbreviations

ANOVA: analysis of variance; DMEM/F12: Dulbecco’s modified Eagle's medium (DMEM), DMEM/Ham's nutrient mixture F12; DMSO: dimethyl sulfoxide; ELISA: enzyme-linked immunosorbent assay; FBS: fetal bovine serum; HPLC/ESI-MS/MS: high performance liquid chromatography-electrospray ionization mass spectrometry; MTT: 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide; PBS: phosphate-buffered saline; Syn.: synonym