Research Journal of Pharmacognosy (RJP) 9(3), 2022: 61–69
Received: 24 Nov 2021
Final Revision: 12 June 2022
Accepted: 13 June 2022
Published online: 16 June 2022
DOI: 10.22127/RJP.2022.316551.1814

Original article

Cytotoxic and Apoptotic Effects of Flavonoids and a Geraniol Derivative from *Artemisia kermanensis* on Normal and Breast Cancer Cell Lines

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Abstract

Background and objectives: The aim of this study was extraction, isolation, and identification of potent metabolites from aerial parts of *Artemisia kermanensis* Podl."Dermaneh kermani', the Iranian endemic species of Asteraceae family, and finally evaluation the anti-proliferative and apoptotic effects against normal and breast cancer cell lines. **Methods:** Air-dried aerial parts of *A. kermanensis* Podl. were extracted by maceration method with acetone/dichloromethane and then fractionated with MPLC apparatus using a gradient solvent system. The chemical structures of the isolated compounds were determined based on analysis of mass and nuclear magnetic resonance spectra. Anti-proliferative effects of the three compounds were determined by MTT assay against HUVEC, MDA-MB-231 and MCF-7 cells. The apoptotic effects of compounds 2 and 3 were determined by flow cytometry on MCF-7 cells. **Results:** Compound 1 (5,7-dihydroxy-3',4',6-trimethoxyflavone, eupatilin), compound 2 (5,7,3'-Trihydroxy-6,4',5'-trimethoxyflavone) and compound 3 (1-acetoxy-3,7-dimethyl-7-hydroxy-octa-2E,5E-dien-4-one) were isolated from the plant extract. Compound 3 was identified for the first time from *A. kermanensis*. Statistical studies revealed that this compound showed the most anti-proliferative properties on all three cell lines. In MCF-7 cells treated with concentration of 10 µg/mL of compound 3, 36% apoptosis was observed. Furthermore, compound 2 (5,7,3'-Trihydroxy-6,4',5'-trimethoxyflavone) showed 33% apoptosis at 650 µg/mL. **Conclusion:** In the present study, the cytotoxic and apoptotic effects of 5,7,3'-trihydroxy-6,4',5'-trimethoxyflavone and Compound 3 (1-acetoxy-3,7-dimethyl-7-hydroxy-octa-2E,5E-dien-4-one) were determined for the first time. On the other hand, since Compound 3 showed the most cytotoxic effects on the cancer cell lines, investigation of its biological effects is recommended.

Keywords: apoptosis; *Artemisia kermanensis*; breast cancer; flavone; geraniol derivative

Citation: Kazemi E, Shafiee F, Yazdiniapour Z. Cytotoxic and apoptotic effects of flavonoids and a geraniol derivative from *Artemisia kermanensis* on normal and breast cancer cell lines. Res J Pharmacogn. 2022; 9(3): 61–69.

Introduction

Cancers occur because of uncontrolled proliferation of abnormal cells, which lead to various types of malignances including carcinoma, leukemia, lymphoma, myeloma, and cancers of the central nervous system [1,2]. Various factors such as some infections, alcohol consumption, tobacco use, radiation exposure, poor nutrition, some internal factors such as hormones, immune system status and hereditary mutations can cause cancer [3]. About 19.3 million new cancer cases and about 10 million deaths due to cancer occurred in 2020 worldwide. Breast cancer with a prevalence of 3.2 million new cases (11.7%) is ranked first in terms of

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prevalence. In the female population, one in four cases of cancer and one in six deaths from cancer are due to breast cancer [4].

The usual treatments for breast cancer including surgery, radiotherapy, chemotherapy, and hormone therapy are not completely effective [5,6]. They also have many side effects such as nausea, fatigue, hair loss, etc. [7]. Various herbal compounds have been used to treat breast cancer with suitable effectiveness, such as paclitaxel from *Taxus baccata* L., etoposide from *Podophyllum peltatum* L., and vinblastin, vinorelbine from *Vinca rosea* L. [8].

Plants of Asteraceae (Compositae) family, with more than 2300 species, are of great morphological diversity and have shown attractive various properties [9]. *Artemisia* is one of the most significant and the largest genus in the Asteraceae family, with more than 400 species [10]. About 34 species of *Artemisia* grow in Iran and two species (*A. kermanensis* Podl., *A. melanolepsis* Boiss) are endemic to Iran [11,12]. *Artemisia* genus contains a wide range of metabolites with diverse structures like phenylpropanoids, flavonoids, terpenes, sterols, lignans, phenolics, fatty acids, fatty esters hydrocarbons, and miscellaneous compounds [10,13]. Various species of *Artemisia* have long been used for medicinal and food purposes. *A. annua* L., for example, has traditionally been used to treat fever and chills in China [14]. Also, several properties such as anti-malarial, anti-inflammatory, antioxidant, and anti-cancer effects have been reported [10,15]. The established cytotoxic effects of various compounds extracted from *Artemisia* species on different cell lines have been reported [16,17]. Furthermore, flavonoids extracted from *Artemisia* have shown cytotoxic effects against several cell lines [18]. Eupatilin, for example, has shown a variety of effects such as anti-inflammatory, antioxidant, anti-tumor, radical scavenging, and anti-allergy activities [19,20]. As an acyclic monoterpene, geraniol showed several biological properties like antimicrobial and anticancer effects [21].

In this study, we aimed to evaluate the cytotoxic and apoptotic effects of three pure compounds extracted from *Artemisia kermanensis* for the first time on HUVEC, MCF-7, and MDA-MB-231 cell lines.

Material and Methods

Ethical considerations

The Ethics Committee of Isfahan University of Medical Sciences approved this research with the code of IR.MUI.RESEARCH.REC.1399.636 on 2021-01-09.

Cell lines and chemicals

HUVEC, MDA-MB-231, and MCF-7 cell lines were purchased from the National Cell Bank of Iran (Pasteur Institute, Tehran, Iran). Annexin-V-FLUOS Staining Kit was obtained from Invitrogen® Company (USA). Trypsin, fetal bovine serum, culture media and DMSO were purchased from Biosera Company (France)**.**

Plant material

Artemisia kermanensis Podl. (Dermaneh) was collected near Taftan volcano in Sistan and Baluchestan province, Iran in the flowering stage in September 2018. A voucher specimen (No. 4001) was deposited at the Herbarium of Dr. Samsam Shariat, Department of Pharmacognosy, Faculty of Pharmacy, Isfahan University of Medical Sciences, Isfahan, Iran.

Extraction

Air-dried samples (whole part) of *Artemisia kermanensis* Podl. (5 kg, dried weight) were extracted by maceration method with acetone/dichloromethane 2:1 solvent (20 L) at room temperature for three days repeated for three times. The extracted solution was filtered and evaporated by a rotary apparatus at a temperature of 40 °C.

Fractionation and isolation

The concentrated extract was chromatographed by MPLC (medium-pressure liquid chromatography) performed on a Büchi 861 apparatus using silica gel (15-40 mesh) as the stationary phase with a gradient solvent system from n-hexane 100% to ethyl acetate 100% by 50 mL fraction volume. The fractions were analyzed by TLC (thin layer chromatography). Similar fractions were mixed together and concentrated under vacuum. TLC (silica gel, hexane: acetone 7:3, v/v) and preliminary ^{1}HNMR (Bruker 400 and $CDCL_3$ as solvent) analysis indicated Fr 2 that was eluted with hexane/ ethyl acetate 30 % contained a series of flavonoids which were separated by further chromatographic procedure. In the previous study, Compound (1) was

determined to be as (5,7-dihydroxy-3 ', 4', 6-trimethoxyflavone: eupatilin) and compound (2) (5,7,3'-Trihydroxy-6,4 ', 5'-trimethoxyflavone) [22]. In this study, Fr 1 (hexane/ ethyl acetate 20 %) was selected for terpenoid isolation using normal phase HPLC (Waters 515 apparatus equipped with a refractive index detector (Waters 2414) using Waters Spherisorb ® Sil 60 (5 µm) (250 mm, 10 mm) column with hexane–EtOAc. Fr 1 that was eluted with hexane–EtOAc (8:2) was purified with hexane–EtOAc (90:10) as the mobile phase, affording compound (3). Structure elucidation of compound (3) was done by extensive spectroscopic analysis, including NMR and GC-MS (Agilent 5975C mass selective detector coupled with an Agilent 7890A GC, equipped with an HP-5MS capillary column (30 m × 0.25 mm; film thickness 0.25 µm) experiments.

Cytotoxicity assay

An appropriate amount of each compound was weighed, and stock concentrations were prepared using DMSO and PBS. Before each MTT test, PBS was used for serial dilution in order to prepare various final concentrations of 400, 200, 100, 50, 25, and 12.5 µg/mL.

Cell suspension with a final density of 3×10^4 cells/mL was prepared from MCF-7 and MDA-MB-231 cell lines using RPMI-1640 cell culture medium and the same number of cells of HUVEC cell line using DMEM cell culture medium enriched by 10% (v/v) of FBS and antibiotics (100 IU/mL penicillin and 100 µg/mL streptomycin). Cell suspension (180 µL) was poured into each well of a 96-well plate, and the wells of the first raw were filled with 200 µL of cell-free culture medium as blank. The plate was then placed at 37 ºC in a CO_2 incubator for 24 hours. Then, 20 µL of various serial dilutions of purified compounds were added to the wells. PBS was used for four wells as the negative control in the same volume and cisplatin as the positive control for all surveyed cell lines. After 24, 48, and 72 hours of incubation, 20 µL of MTT solution (5 mg/mL) was added to all wells, and incubation was performed for another 3 hours. Finally, by adding 150 µL of DMSO to the wells, the formazan crystals were dissolved and the absorbance at 570 nm was determined using a microplate reader (BioRad, USA) [23].

Determining the mechanism of cell death using flow cytometry

About 3×10^5cells of MCF-7 cell line in 900 µL of RPMI-1640 were added to the wells of 6-well plates and incubated for 24 h. Then, the purified compounds were added to the related wells in two concentrations around the IC_{50}. Incubation was performed for 24 h and the cells were detached using trypsin and poured into the flow cytometry tubes. The cells were then washed twice with PBS and centrifuged. The supernatant was removed and 200 µl of 1X Binding Buffer was added to all tubes. Sample treatment with PI (propidium iodide) and AnnexinV-FITC-Conjugated was performed according to the Kit manufacturer manual (eBioscience™ Annexin V-FITC Apoptosis Detection Kit) and incubated for 15 min at room temperature with protection from light. Finally, dilution was performed with 300 µL of 1X Binding Buffer and under-went to the flow cytometry apparatus (BD FACSCalibur, USA).

Statistical analysis

The results were obtained from three repetitions of the experiment and four replicate wells for each concentration. The results were reported as mean±SD. The final results were evaluated using SPSS software (version 23) (USA) using one-way ANOVA statistical test with a significance level of $p<0.05$. Tukey post hoc test was used to determine the differences between groups. At the end, the IC_{50} value of each investigated sample was determined by drawing the graph of cell survival percent against concentration using GraphPad Prism 7.0 software.

Results and Discussion

Compound 3 was obtained as a pale-yellow oil. ^{1}H NMR (400 MHz, CDCl3) δ ppm 6.94 (d, J=15.2 1 H-6), 6.87 (d, J=15.2 1 H-5), 6.61 (t, J=6.4 Hz, 1 H-2), 4.82 (d, J=6.4 Hz, 2 H-1), 2.11 (s, 3 H-2'), 1.88 (s, 3 H-10), 1.39 (s, 3 H-8), 1.39 (s, 3 H-9). C NMR (100 MHz, CDCl3) δ ppm 191.7 (C-4), 170.9 (C-1'), 153.7 (C-6), 139.8 (C-3), 135.5 (C-2), 120.5 (C-5), 71.5 (C-7), 61.5 (C-1), 29.7(C-8), 29.7 (C-9), 21.0(C-2'), 12.3(10). MS (GC) m/z 208.1 $[M-H_2O]^+$ and 167.1 $[M-OAc]^+$. The molecular formula $C_{12}H_{18}O_4$ *m/z* 226.2 was suggested on the basis of NMR and MS data and the structure was determined as 1-acetoxy-3,7-dimethyl-7-hydroxy-octa-2E,5E-dien-4-one a highly oxygenated geraniol derivative (Figure 1). Numerous studies have been performed to investigate the effects of various extracts and compounds from *Artemisia* species on different cell lines.

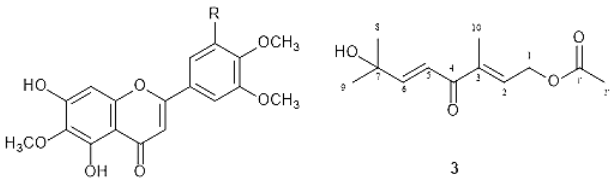

Figure 1. Chemical structures of compounds 1-3

The results of the MTT assay in our study showed that approximately all surveyed compounds revealed their cytotoxic effects concentration-dependently, the higher the concentration of the compounds, the greater the observed cytotoxic effects.

For MCF-7 cells, according to the calculated IC_{50}, it was concluded that over time, the cytotoxicity of each compound increased. For example, for compound 1, there were significant differences between the IC_{50} value after 24 and 48 hours (p-value = 0.007) and also between the 48- and 72-hours' time of incubation (p-value = 0.003) (Figure 2a).

For MDA-MB-231 cell line in this regard, for compound 1, for example, the decrease in the IC_{50} value was confirmed. However, for compound 2, there were no significant differences between the cytotoxic effects in the highest surveyed concentration after 48 and 72 hours of incubation (p-value = 0.78) suggesting that this compound showed its best effects in 48 h (Figure 2b).

Finally, according to the calculated IC_{50}s for the HUVEC cell line, it was confirmed that the IC_{50} of each compound was significantly more than the IC_{50} value for cancer cells in similar conditions (same concentration and same incubation time, with p value = 0.023 and 0.041 in comparison to the MCF-7 and MDA-MB-231, respectively). However, even for the normal cells, the observed cytotoxicity followed the time and concentration dependency (Figure 2c.).

According to the calculated cell survival percent represent in the Figure 2, and calculated IC_{50} value, it was concluded that compound 3 was the most cytotoxic compound for all three cell lines and the cytotoxicity of compound 1 was more obvious than compound 2. Finally, although MCF-7 cells showed more susceptibility to the surveyed samples, there was no significant differences between the MCF-7 and MDA-MB-231 cells in this regard (p value =0.083) (Table 1).

In a related study, the effectiveness of ethyl acetate extract and several compounds that were extracted from *A. indica* on five cell lines was evaluated. Ethyl acetate extract showed cytotoxic effects on MCF-7 cell line with an IC_{50} value of 67.09 µM [24].

In Shafi et al.'s study, the methanolic extract of *A. absintium* aerial parts showed cytotoxic and apoptotic effects on MCF-7 and MDA-MB-231 cell lines with IC_{50} value of 25 and 20 µg/mL, respectively [25]. In the mentioned research, these two cells were used as the estrogen receptor-positive (MCF-7) and negative (MDA-MB-231) cells and based on the calculated IC_{50}s which were in the same amounts, it was concluded that the observed cytotoxic effects were due to the compounds without estrogen receptor inducing activity.

In another similar study, the cytotoxicity of methanol extracts of several parts of five *Artemisia* species including flowers, leaves, stems, and roots was investigated against MCF-7 cancer cells as well as HEK293 normal cells.

Table 1. The calculated IC_{50} of different compounds from *Artemisia kermanensis* against cell lines

Cell line	Incubation Time (h)	IC_{50} (µM)		
		Compound 1	Compound 2	Compound 3
HUVEC	24	291	Unaccountable*	94
	48	166	Unaccountable	60
	72	131	Unaccountable	41
MDA-MB-231	24	280	Unaccountable	83
	48	122	1100	30
	72	99	1045	11.3
MCF-7	24	285	Unaccountable	51.5
	48	119	Unaccountable	33.5
	72	87	Unaccountable	20.7

* IC_{50} was not countable in investigated concentrations

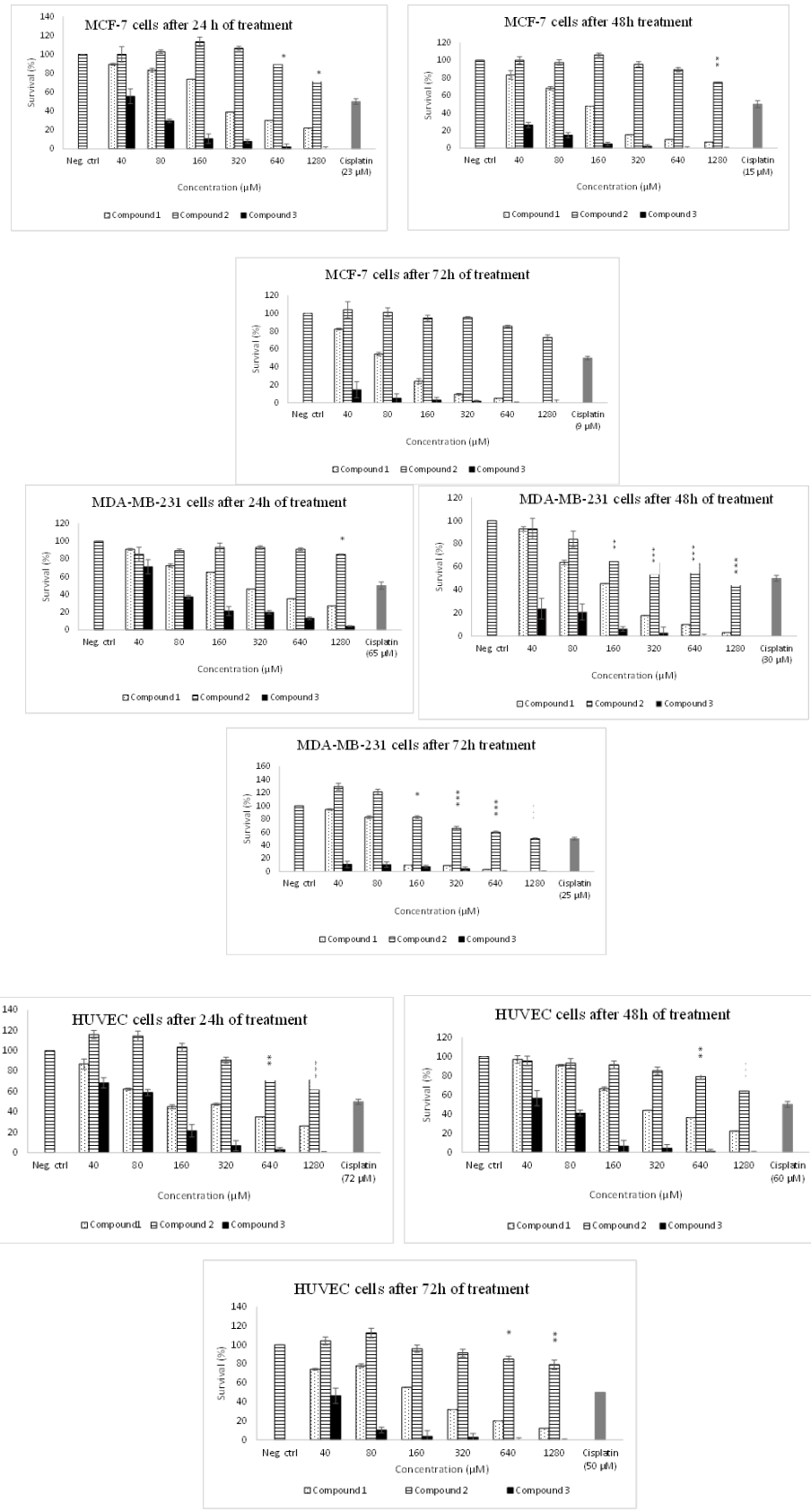

Figure 2. Cell survival of various cell lines after their treatment with compounds 1-3 by increasing time of incubation, 24, 48, and 72 hours; cisplatin was used as the positive control; negative control was PBS (the dilution solvent); * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ show the significant difference between compound (2) in comparison to the control cells; n = 3

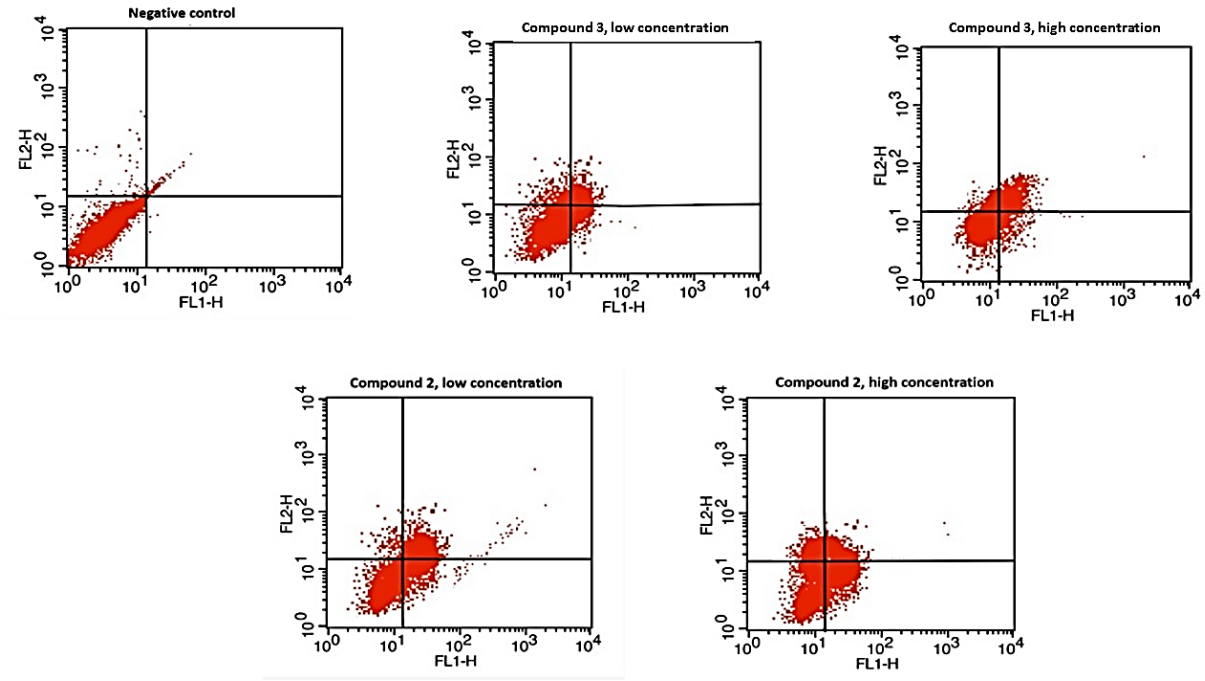

Figure 3. Flow cytometry assay of MCF-7 cells treated with two concentrations of compound (2) (2.9 and 5.8 mM) and compound (3) (8 and 28 µM) from *Artemisia kermanensis.* Lower left chamber: live cells (annexin V^-/ PI^-); lower right chamber: early apoptotic cells (annexin V^+/ PI^-); upper left chamber: dead cells (annexin V^-/ PI^+); upper right chamber: late apoptotic cells (annexin V^+/ PI^+)

The results showed that flower extract of *A. absinthium* (IC_{50}=221.5 µg/mL) and *A. vulgaris* (IC_{50}=500 µg/mL) inhibited the MCF-7 cell line more than others.Also, various extracts of *A. spicigera* and *A. fragrans* did not show cytotoxic effects and even increased the number of cells [26]. These findings reinforce the fact that the observed cytotoxic effects might be due to the flavone existing in the flower than other parts of the plant.

In one study on the investigation of the cytotoxic effects of eupatilin, a concentration- and time-dependent inhibitory effect was established on H-ras-transformed human breast epithelial cells (MCF10A-ras). When these cells were treated with 100 µM of eupatilin, DNA synthesis was inhibited in a concentration-dependent manner [27]. At these concentrations, the cytotoxic effects of two investigated purified flavone in our study was less than 5,7-dihydroxy-3',4',6 – trimethoxyflavone with the same backbone. The most probable reason for this observation is the difference in cell susceptibility.

To compare with the cytotoxic effects of other flavones; for example, apigenin isolated from *A. annua* at a concentration of 40 µM showed apoptotic effects on A2780 cells (ovarian cancer cell line) and stopped the cells at the G2/M stage. Eupatorin also inhibited MDA-MB-468 cell growth in a concentration-dependent manner with IC_{50} of 0.5 µM [28].

The second part of this study was to determine the cell death mechanism of compound 3 as the compound with the most anti-proliferative effects and compound 2 as a new structure from *A. kermanensis* with no previous biological report. The MCF-7 cell analysis by flow cytometry showed about 96% of survived cells, 3% apoptosis, and 1% necrosis in the negative control sample. For the cells treated with 8.3 µM of compound 3, 60% of cells survived, 28% of them showed apoptosis, and 12% necrosis was determined. These percent for cells treated with 28 µM of compound 3 were about 50% cell survival, 36% apoptosis, and 14% necrosis. For the cells encountered with 2.9 mM of compound (2), 63% cell survival, 33% apoptosis, and 4% necrosis were determined. Finally, in cells treated with a concentration of 5.8 mM of compound (2), 59% cell survival, 25% apoptosis, and 16% necrosis in MCF-7 cells were recorded.

There are some related studies; for example, in a

study synthetic eupatilin induced apoptosis in HCT116 and HT29 cells in concentrations of about 50-100 µM after 48 hours of incubation [29]. Furthermore, the natural eupatilin extracted from *A. asiatica* showed apoptosis induction in LLC-PK1 kidney cells in 50 µg/mL after 24 h of treatment as about 50 % [30]. Although, we observed the cytotoxic and apoptotic effects of purified flavones in higher concentrations, a non-significant reduction in the survival of normal cells was also established.

So far, no work has been done on compound 3; Only in 1987 in the Rustaiyan et al. study, this compound was extracted from *A. aucheri* Boiss aerial parts [31]. Studies have been performed to investigate the cytotoxic effects of acyclic monoterpenes. For example, geraniol as a monoterpene, showed good cytotoxic effects on several cancer cell lines, including breast cancer cell lines. Geraniol stopped the growth of MCF-7 cells in the G1 phase; It was not effective on normal breast epithelial cells (MCF-10F), which indicates the specific effect of this compound [32]. Linalool as another terpene with related structure to our investigated compounds (IC_{50} = 224 µM) inhibited the growth of breast cancer cell line (T-47D) and induced apoptosis; this compound also increased the serum level of various cytokines, which could enhance the Th1 cellular immune response in T-47D cells [33]. Citronellol showed cytotoxic effect on MCF-7 cell line (IC_{50} = 80 µM) and MDA-MB-231 (IC_{50} =35 µM); It also induced apoptosis [34]. Therefore, it is not far-fetched to expect anti-apoptotic effects from the compounds extracted from *A. kermanensis* and it can be concluded that similarly, these compounds have the potential to induce mitochondrial-related apoptosis and DNA fragmentation ability apoptosis, which needs more studies.

Conclusions

In the present study, the fractionation and purification of two flavones (compound 1 and 2) and one acetylated geraniol derivative (compound 3), were performed and compound 3 was identified for the first time from *A. kermanesis* The anti-proliferative effects were investigated. Our data confirmed that compound 3 had the most cytotoxic effects and can be used for further investigation. Furthermore, we showed for the first time that compound 2 led to the apoptotic effects on MCF-7 cells. So, it is suggested for more surveys about this compound and other probable biological effects as well as investigation on cytotoxic effects against other cancer cell lines.

Acknowledgments

This paper was taken from the thesis of Elahe Kazemi submitted for fulfillment of the Pharm D in the Faculty of Pharmacy and Pharmaceutical Sciences, Isfahan University of Medical Sciences, Isfahan, Islamic Republic of Iran.

Author contributions

Elahe Kazemi performed the experimental and wrote the manuscript draft; Fatemeh Shafiee revised the manuscript, conducted the biological tests and analysed the data; Zeinab Yazdiniapour revised the manuscript and conducted the plant extraction, fractionation, purification and identification of compounds.

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] Safarzadeh E, Sandoghchian Shotorbani S, Baradaran B. Herbal medicine as inducers of apoptosis in cancer treatment. *Adv Pharm Bull*. 2014; 4(S1): 421–427.

[2] Jena J, Ranjan R, Ranjan P, Sarangi MK. A study on natural anticancer plants. *Int J Pharma Chem Sci*. 2012; 1(1): 365–368.

[3] Anand P, Kunnumakara AB, Sundaram C, Harikumar KB, Tharakan ST, Lai OS, Sung B, Aggarwal BB. Cancer is a preventable disease that requires major lifestyle changes. *Pharma Res*. 2008; 25(9): 2097–2116.

[4] Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, Bray F. Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA: A Can J Clin*. 2021; 71(3): 209–249.

[5] Lopes CM, Dourado A, Oliveira R. Phytotherapy and nutritional supplements on breast cancer. *Bio Med Res Int*. 2017; Article ID 7207983.

[6] Waks AG, Winer EP. Breast cancer treatment: a review. *J Am Med Ass*. 2019; 321(3): 288–300.

[7] Nurgali K, Jagoe R, Abalo R. Editorial: Adverse effects of cancer chemotherapy:

anything new to improve tolerance and reduce sequelae? *Front Pharmacol*. 2018; 9: 245–247.

[8] Richard TS, Kamdje AHN, Mukhtar F. Medicinal plants in breast cancer therapy. *J Dis Med Plants*. 2015; 1(1): 19–23.

[9] Sestak Z, Bohm BA, Stuessy TF. Flavonoids of the sunflower family (Asteraceae). *Biologia Plantarum*. 2002; 2(45): 226.

[10] Koul B, Taak P, Kumayr A, Khatri T, Sanyal I. The *Artemisia* genus: a review on traditional uses, phytochemical constituents, pharmacological properties and germplasm conservation. *J Glyco Lipido*. 2018; 7(1): 1–7.

[11] Mojarrab M, Naderi R, Afshar FH. Screening of different extracts from *Artemisia* species for their potential antimalarial activity. *Iran J Pharm Res*. 2015; 14(2): 603–608.

[12] Rustaiyan A, Tabatabaei-Anaraki M, Kazemi M, Masoudi S, Makipour P. Chemical composition of essential oil of three *Artemisia* species growing wild in Iran: *Artemisia kermanensis* Podl., *A. kopetdaghensis* Krasch., M. Pop et Lincz. ex Poljak., and *A. haussknechtii* Boiss. *J Essen Oil Res*. 2009; 21(5): 410–413.

[13] Adewumi OA, Singh V, Singh G. Chemical composition, traditional uses and biological activities of *Artemisia* species. *J Pharmacog Phytochem*. 2020; 9(5): 1124–1140.

[14] Abad MJ, Bedoya LM, Apaza L, Bermejo P. The *Artemisia* L. genus: a review of bioactive essential oils. *Molecules*. 2012; 17(3): 2542–2566.

[15] Grech-Baran M, Pietrosiuk A. *Artemisia* species in vitro cultures for production of biologically active secondary metabolites. *BioTechnologia J Biotechnol Comput Biol Bionanotechnol*. 2012; 93(4): 371–380.

[16] Ko YS, Lee WS, Panchanathan R, Joo YN, Choi YH, Kim GS, Jung JM, Ryu CH, Shin SC, Kim HJ. Polyphenols from *Artemisia annua* L. inhibit adhesion and EMT of highly metastatic breast cancer cells MDA-MB-231. *Phytother Res*. 2016; 30(7): 1180–1188.

[17] Taleghani A, Emami SA, Tayarani-Najaran Z. *Artemisia*: a promising plant for the treatment of cancer. *Bioorg Med Chem*. 2020; 28(1): 115180–115201.

[18] Yuan H, Lu X, Ma Q, Li D, Xu G, Piao G. Flavonoids from *Artemisia sacrorum* Ledeb. and their cytotoxic activities against human cancer cell lines. *Experiment Thera Med*. 2016; 12(3): 1873–1878.

[19] Son JE, Lee E, Seo SG, Lee J, Kim JE, Kim J, Lee KW, Lee HJ. Eupatilin, a major flavonoid of *Artemisia*, attenuates aortic smooth muscle cell proliferation and migration by inhibiting PI3K, MKK3/6, and MKK4 activities. *Planta Med*. 2013; 79(12): 1009–1016.

[20] Yu K, Li XM, Xu XL, Zhang RY, Cong HL. Eupatilin protects against tumor necrosis factor-α-mediated inflammation inhuman umbilical vein endothelial cells. *Inter J Clin Experiment Med*. 2015; 8(12): 22191–22197.

[21] Mączka W, Wińska K, Grabarczyk M. One hundred faces of geraniol. *Molecules*. 2020; 25(14): 1–16.

[22] Yazdiniapour Z, Yegdaneh A, Akbari S. Isolation and characterization of methylated flavones from *Artemisia kermanensis*. *Adv Biomed Res*. 2021; 10(1): 1–4.

[23] Riss TL, Moravec RA, Niles AL, Duellman S, Benink HA, Worzella TJ, Minor L. Cell viability assays. Assay Guidance Manual [Internet]. [Accessed 2022]. Available from https://www.ncbi.nlm.nih.gov/books/NBK144065/

[24] Zeng YT, Jiang JM, Lao HY, Guo JW, Lun YN, Yang M. Antitumor and apoptotic activities of the chemical constituents from the ethyl acetate extract of *Artemisia indica*. *Mol Med Rep*. 2015; 11(3): 2234–2240.

[25] Shafi G, Hasan TN, Syed NA, Al-Hazzani AA, Al-shatwi AA, Jyothi A, Munshi A. *Artemisia absinthium* (AA): a novel potential complementary and alternative medicine for breast cancer. *Mol Biol Rep*. 2012; 39(7): 7373–7379.

[26] Gordanian B, Behbahani M, Carapetian J, Fazilati M. In vitro evaluation of cytotoxic activity of flower, leaf, stem and root extracts of five *Artemisia* species. *Res Pharma Sci*. 2014; 9(2): 91–96.

[27] Kim DH, Na HK, Oh TY, Kim WB, Surh YJ. Eupatilin, a pharmacologically active flavone derived from *Artemisia* plants, induces cell cycle arrest in ras-transformed human mammary epithelial cells. *Biochem Pharmacol*. 2004; 68(6): 1081–1087.

[28] Ferreira JF, Luthria DL, Sasaki T, Heyerick A. Flavonoids from *Artemisia annua* L. as antioxidants and their potential synergism with artemisinin against malaria and cancer.

Molecules. 2010; 15(5): 3135–3170.

[29] Lee M, Yang C, Song G, Lim W. Eupatilin impacts on the progression of colon cancer by mitochondria dysfunction and oxidative stress. *Antioxidants*. 2021; 10(6): 957–973.

[30] Park JY, Lee D, Jang HJ, Jang DS, Kwon HC, Kim KH, Kim SN, Hwang GS, Kang KS, Eom DW. Protective effect of *Artemisia asiatica* extract and its active compound eupatilin against cisplatin-induced renal damage. *Evid Base Complem Alt Med*. 2015; Article ID 483980.

[31] Rustaiyan A, Bamonieri A, Raffatrad M, Jakupovic J, Bohlmann F. Eudesmane derivatives and highly oxygenated monoterpenes from Iranian *Artemisia* species. *Phytochemistry*. 1987; 26(8): 2307–2310.

[32] Cho M, So I, Chun JN, Jeon JH. The antitumor effects of geraniol: modulation of cancer hallmark pathways. *Int J Oncol*. 2016; 48(5): 1772–1782.

[33] Chang MY, Shen YL. Linalool exhibits cytotoxic effects by activating antitumor immunity. *Molecules*. 2014; 19(5): 6694–6706.

[34] Rajendran J, Pachaiappan P, Thangarasu R. Citronellol, an acyclic monoterpene induces mitochondrial-mediated apoptosis through activation of proapoptotic factors in MCF-7 and MDA-MB-231 human mammary tumor cells. *Nutr Cancer*. 2021; 73(8): 1448–1458.

Abbreviations

HUVEC: human umbilical vein endothelial cell; IC_{50}: 50% inhibitory concentration; MPLC: medium performance liquid chromatography; HPLC: high performance liquid chromatography; TLC: thin layer chromatography; NMR: nuclear magnetic resonance; GC-MS: gas chromatography mass spectrometry