Chemical Characterization and Evaluation of Anticancer Effect of *Falcaria vulgaris* via Induction of Apoptosis

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Research Article

Keywords: F. vulgaris, Cancerous cells, GC-MS, Cytotoxicity, Apoptosis, Volatile compounds

Posted Date: June 21st, 2021

DOI: https://doi.org/10.21203/rs.3.rs-530162/v1

Abstract

Background: Apiaceae family is one of the plant families which used for medical investigation. Falcaria vulgaris is a clear example of this genus that grows in certain regions of Iran. In traditional medicine, due to the presence of coumarin and flavonoid compounds in this plant, therapeutic properties such as gastrointestinal and liver diseases, skin ulcers, gastric ulcers and intestinal inflammation have been reported. It has also been found that these compounds lead to cytotoxic effects.

Objective: The aim of this study aimed to investigate the cytotoxic effect and induction of apoptosis by various extracts and essential oil of F. vulgaris on cancerous cell (SW-872) and to identify the volatile compounds of effective samples.

Methods: The shoot of the plant was extracted by Soxhlet apparatus and its essential oil was taken by Clevenger apparatus. The cytotoxicity of the samples was evaluated by MTT method and the mechanism of cancer cell death by flow cytometry and finally, the volatile compounds of essential oils and effective extracts were identified by GC-MS. Results: The results showed that n-Hexane extract and 40% VLC fraction had the greatest cytotoxic effect on SW-872 cells. While, the most abundant volatile compounds in essential oil and 40% VLC fraction of n-Hexane extract are terpenoid compounds like (+) spathulenol and caryophyllene oxide, in n-Hexane extract tetradecan, and spathulenol are the most, respectively.

Conclusion: In sum, it was found that the fraction of 40% n-Hexane is in a concentration-dependent manner and significantly with controlling cells, inhibit the growth of cancer cells. This effect is through induction of apoptosis and due to the presence of effective volatile compounds such as terpenoids and non-terpenoids which can be considered as the valuable natural sources for the isolation of anti-cancer compounds.

Introduction

Cancer is a disease in which the growth and proliferation of cells increase uncontrollably and can spread to other parts of the body [1] In 2020 in the United States, about 1.8 million new cases of cancer were reported, of which about 606 died [2]. So far, effective therapies for the prevention and treatment of cancer, including immunotherapy, hormone therapy, radiotherapy, chemotherapy and surgery, have been reported [3]. Despite extensive research into cancer and the discovery of related treatments, it is still considered a scary disease [4]. On the other hand, after years of progress in the field of health, today developing countries are involved in the fight against deadly cancers [3]. At present, nonspecific function, drug resistance and severe side effects are among the main problems of chemotherapy drugs. Therefore, there is an urgent need for extensive studies to find alternative and complementary therapies [5]. Using high-performance screening, researchers have found that a variety of natural products, such as herbs, can be used as therapeutic agents in chemoprevention [6]. Scientific information on the safety and effectiveness of herbal remedies has shown that these plants have good anti-cancer activity and most of them are currently under clinical trial [7-9].

The compounds in plants play a key role in killing malignant cancer cells through the apoptotic pathway [10, 11]. Apoptosis or programmed cell death is a defense mechanism that occurs due to molecular events and changes in cell morphology such as blabbing, cell shrinkage, nuclear fragmentation, chromatin condensation, and so on [12]. Therefore, many researchers have tried to discover therapeutic agents to stimulate malignant cancer cells by apoptosis, to offer a new treatment strategy [13]. In this regard, many studies have reported the therapeutic properties of plants of the *Apiaceae* family, especially the genus *Falcaria* (*F. vulgaris*) [14-16]. The plants of this family are widely used in traditional Iranian medicine and have antimicrobial effects [14], treatment of gastrointestinal diseases [17, 18], skin diseases [19], anti-fertility [20], analgesia [21, 22], treatment of heart diseases [23], etc.

Among the effective compounds of plants of this family is Spathulenol, Carvacrol, Germacrene-B, α-pinene, α-Terpinyl acetate and β-caryophyllene [24-28]. The plants of this family are mainly distributed in the northern, northwestern and western regions of Iran and grow mostly in East Azarbaijan province [29]. The aim of the present study was to investigate the cytotoxic effects of *F. vulgaris* on SW-872 (skin cancer) cancer cell line.

Materials And Methods

Chemicals:

Substances such as n-Hexane, dichloromethane, methanol and ethyl acetate (Caledon Canada), RPMI1640, FBS, Penicilin/Streptomycin (Gibco™ Invitrogen, USA) and MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) (Sigma-Aldrich, USA) were purchased.

Plant collection and drying:

F. vulgaris plants were collected in the spring of 2016 from Moghan region located in Ardabil province. After identification by a plant expert, its herbarium specimen with number Tz fph 178 was kept in the herbarium of Tabriz University of Medical Sciences. After washing the collected samples, the shoots of *F. vulgaris* were completely dried in the open air and laboratory conditions and then ground into a fine powder by an electric mill.

Extraction and fractionation:

First, 200 g of *F. vulgaris* plant powder was weighed and after loading in a suitable filter paper, it was placed in a 1 liter Soxhlet apparatus and extracted with N-Hexane (n-Hex), dichloromethane (DCM) and methanol (MeOH) solvents, respectively, for 72 hours. The extracts were then dried completely by rotary evaporator at 45°C under low air pressure and finally weighed. Effective non-polar extracts were fractionated using VLC method with the stationary phase of silica gel, n-Hex solvents and increasing percentages of ethyl acetate. Extracts and fractions were stored frozen until use.

Essence extraction:

The choice of essential oil extraction method depends on the type and condition of the plant, the active ingredients available and the purity of the final product. The water distillation process is used on dry plants to prevent the destruction of compounds due to the high boiling temperature of the water. In essential oil distillation, 100 g of plant shoot powder with 150 ml of distilled water and 50 ml of glycerin were poured into a 1-liter round bottom balloon. The balloon was then connected to a clevenger device and placed in a mantel heater to provide the necessary heat to penetrate the plant tissues. Glycerin also helps to extract essential oils from plant powder by increasing the permeability of plant cell walls. A Clevenger device with a functional design is the most suitable tool for cooling the vapors emitted from the sample and extracting the resulting essential oil. The essential oil extraction process was performed for 4 hours according to the British Pharmacopeia method. Finally, the essential oil was then collected in a microtube and stored in a refrigerator.

Identification of volatile compounds in non-polar samples:

Gas chromatography-mass spectrometry (GC-MS) was used to identify compounds in essential oils and extracts of n-Hex and its VLC fraction of n-Hex extract. GC-MS analysis was performed on a Shimadzu GC-MS QP 5050A gas chromatograph-mass spectrometer fitted with a fused capillary column DB-1 (60 m, 0.25 mm id, film thickness 0.25 µm) [30]. Helium was utilized as the carrier gas, at a flow rate of 1 ml/min.

Cell culture:

SW-872 (Human liposarcoma cell line) and HFFF (Normal human skin fibroblasts) cells were obtained from the Pasteur Institute of Iran and grown in RPMI-1640 medium containing 10% FBS supplemented with 1% antibiotic (penicillin/streptomycin) at 37°C in a humidified, 5% CO_2 incubator.

Preparation of plant samples:

Diluted samples of plant extracts are needed to perform cytotoxicity tests and observe the testable result. For this purpose, 10 mg of each dried extract was carefully weighed and completely dissolved in separate microtubes in 100 µl DMSO; then the contents of each microtube were brought to a volume of 1 ml using 900 µl of complete culture medium. Due to the concentration of active herbal substances in the fractions of each extract, their diluted samples were prepared with 0.1 concentration of total extracts. Therefore, the exact amount of 1 mg of each fraction was dissolved in 10 µl of DMSO and brought to a volume of 1 ml.

MTT assays:

MTT assay was used for determination of substances toxicity on cell lines and evaluating of IC_{50} Cell suspension with a concentration of 3×10^4 cells per ml was prepared and inoculated in 96-well microplates for 24 hours. The appropriate amount of extracts and fractions were added to control and test wells and the plates were incubated for 24 and 48 hours. Moreover, 20 µl of MTT solution was added

to each well and incubated for 3 hours. The supernatant was replaced with 150 microliters of DMSO and dissolved the formazan crystals. Finally, it was measured by the ELISA plate reader at 570 nm (Anthos, Austria).

Detection of apoptosis by flow cytometry:

At this stage, the Annexin-V-Fluos Staining kit was used, which contains both AnnexinV material (to bind to the phospholipid molecule) and PI dye, which makes it possible to identify and clean apoptotic cells from necrotic cells. For flow cytometric testing, SW-872 cells were distributed in 6 plates so that each well contained 2×10^5 cells. After 24 hours of incubation, the cells were treated with IC_{50} concentrations of fractions in pairs. Then the cells were isolated from the bottom of the well and transferred to separate microtubes. After centrifugation, the supernatant is removed and the cells are washed with 500 µl of PBS and centrifuged again. the washed cells of each microtube are slowly dispersed in 100 µl of diluted Annexin V Binding buffer and 5 µl of Annexin solution and PI dye is added to each microtube and incubated in the dark for 30 minutes at room temperature. After this time, the cells are centrifuged and after removing the supernatant, they are dispersed in 200 µl of Annexin V Binding buffer. Finally, the absorption intensity and fluorescence of the samples are read using a flow cytometer at 488 wave lengths.

Statistical analysis:

In the present study, all experiments were repeated at least twice and the results were obtained as mean ± standard deviation by descriptive statistics. Statistical analyzes were performed using Graph pad prism 8 software. For comparison between groups, ANOVA and Tukey post hoc test were used and the minimum significance level was considered $p < 0.05$.

Results

Amounts of extracts and fractions:

The amount of extracts obtained from one extract of 200 g of aerial part powder of *F. vulgaris* plant using Soxhlet method and solvents of n-Hex, DCM and MeOH is shown in Table 1. In addition, the results of fractionation of 2 g of n-Hex extract by VLC method with n-Hex solvents and increasing percentages of ethyl acetate and silica gel stationary phase are shown in Table 1.

Results of cytotoxicity on SW-872 cell line:

The results of MTT testing of n-Hex, DCM and MeOH extracts on SW-872 cells at 24 and 48 hours. According to the results, we observed the remarkable cytotoxicity of n-Hex extract on SW-872 cells, compared to other extracts and DMSO control; therefore, in the next step, n-Hex fractions were tested by MTT. The results of this test at 24 and 48 hours are shown in Figure 1.

The IC Wesołowska values calculated from the Non-linear regression method is as shown in Table 2. To compare the cytotoxic effect of different plant samples at 24 and 48 hours, Two Way ANOVA test and TUKEY post hoc test were performed to compare the effect of different groups together with DMSO control. The results show that all samples had a significant cytotoxic effect compared to DMSO control. In statistical tests, the significance level was defined as ns ($p > 0.05$), * ($p < 0.05$), ** ($P < 0.01$), *** ($p < 0.001$), **** ($P < 0.0001$) Becomes. A significant comparison of the effect of different samples with controls is presented in Figure 2.

Results of cytotoxicity on HFFF cells:

To investigate the effect of cytotoxicity of *F. vulgaris* extracts and fractions on non-cancerous cells, MTT assay was performed on normal HFFF cell lines. Table 3 shows the IC50 results of 24- and 48-hour treatments on these cells.

Apoptosis test results in SW-872 cells:

Flow cytometry test was performed to evaluate the mechanism of cell death induced by DCM extract fractions on SW-872 cells and the results are shown in Figure 3. The results showed that the 20% and 40% fractions of n-Hex extract have the highest induction of apoptosis on SW-872 cells and have a suitable cell death mechanism.

Identification of compounds in essential oil using gas chromatography-mass spectrometry: Using the GC-MS device, the compounds in the volatile oil of *F. vulgaris* were identified, which are shown in Table 4 with specifications such as inhibition time, percentage of constituents and KI.

Identification of compounds in n-Hex extract:

Data on the compounds identified in the n-Hex extract of *F. vulgaris* obtained by injection into the GC-MS are plotted in Table 5

Identification of compounds in 40% VLC fraction of n-Hex extract:

The data on the compounds identified in the 40% VLC fraction of n-Hex extract of *F. vulgaris* obtained by injection into the GC-MS device are presented in Table 6.

Evaluation of cytotoxicity of volatile oil:

The results of cytotoxicity of *F. vulgaris* volatile oil on SW-872 cancer cells in 24-hour treatment are 3.78±0.93 which found that the highest toxicity effect occurred on SW-872 cell line.

Discussion

According to the World Health Organization (WHO), 9.6 million deaths in 2018 worldwide will be due to various types of cancer, and the number of these deaths is projected to reach more than 11 million per

year by 2030 [31]. Today, the world is facing a high prevalence of cancer, which is the second leading cause of death after heart disease. Understanding the important mechanisms involved in causing cancer is important for advancing therapies for the treatment of neoplasms [32]. Chemotherapy drugs should ideally have a specific cytotoxic effect on neoplastic cancer cells; however, in reality this treatment leads to some systemic toxicity to the individual [33]. Apoptosis, the best programmed cell death, is involved in controlling the number of normal cells and their proliferation as part of the natural development process [34].

75-80% of the world's populations, especially in developing countries, use herbal medicines to treat diseases. This is because they believe that herbal medicines, in addition to being cheap and available, have fewer side effects. Many common medicines are derived from plant sources. In the past, the basis of many drugs was herbal, including aspirin (bark willow), digoxin (fox glove) and morphine (opium poppy), etc [35]. Various studies have shown that plant compounds play an important role in both the prevention and treatment of cancers. These compounds work by different mechanisms; however, the induction of apoptosis is a common point of many of these compounds [36]. The effect of coumarins on the cytotoxic effect of members of the Apicaceae family has also been proven in studies [37-39]. The significant antioxidant effect of *F. vulgaris* due to the presence of compounds such as alkaloids, anthraquinones, flavonoids, phenols, saponins, steroids and tannins has already been investigated [24]. Antioxidant compounds such as Spathulenol and Carvacrol have also been identified as the most essential oils of the plant [25]. In the present study, the cytotoxic effects of n-Hex, DCM and MeOH extracts of *F. vulgaris* on SW-872 (skin cancer) and HFFF cell lines were investigated for the first time. In this regard, at the beginning of the work, cytotoxicity and IC_{50} values obtained from the treatment of these cells with different extracts were investigated. According to evaluate the cytotoxicity of the plant on human liposarcoma cells (SW-872), MTT assay was performed with three extracts of n-Hex, DCM and methanol. MeOH extract had no notable effect on cell growth and n-Hex extract had the most cytotoxic effect compared to DMSO control ($p < 0.0001$) and DCM extract, at time 24 ($p < 0.001$) and 48 hours ($P < 0.01$). Flow cytometric results of this extract showed that the mechanism of cell death induced by it was both apoptosis and necrosis. In order to further investigate and isolate the apoptosis-inducing compounds, n-Hex extract was selected for further study. The results of cytotoxicity of n-Hex extract fractions on SW-872 show that the anticancer effect of all fractions in 24 and 48 hours treatment is significantly different from the control ($p < 0.0001$). The 40% and 60% fractions have the best IC_{50} values among the samples so that the cytotoxic effect of these two samples is not significantly different from each other. 40% fraction showed the highest amount of cytotoxicity which is significantly different from the effect of other fractions ($p < 0.05$).

The essential oil components of this plant, which were collected from Moghan region in spring, are abundant in the category of compounds with the structure of oxygenated sesquiterpene (75.3%), sesquiterpene hydrocarbon terpenes (10.8%), hydrocarbon monoterpenes (0.6%) were found and the total terpene content of essential oil was 86.53%. The results also showed that spathulenol (33.8%) and caryophyllene oxide (18.3%) are the most abundant volatile compounds of *F. vulgaris,* respectively. The

yield of volatile oil was determined as 0.206% by volume/weight. Caryophyllene oxide has a considerable cytotoxic effect on different cancer cells that is dose and time dependent. The best reported IC_{50} value of caryophyllene oxide isolated from *Psidium cattleianum* is 3.95 ± 0.23 μM [40]. Spathulenol has also been suggested as a good candidate for the treatment of drug resistance in cancer treatment [41]. Therefore, the effect of remarkable cytotoxicity ($p<0.0001$) of *F. vulgaris* essential oil than DMSO control on cancer cells can be considered due to the presence of valuable anti-cancer compounds such as caryophyllene oxide and spathulenol. Another species of this plant that grows in Iran is *Falcaria falcariodes*. The results of a study conducted by Dr. Masoudi *et al.* on this plant identified 24 compounds that make up 97.6% of the total volatile oil of the plant; Among these compounds, germacrene B (67.9%) is the main compound of this plant [26].

The most probable compounds identified by GC-MS method of n-hexane extract are non-terpenoid compounds, most of which include tetradecane (17.64%), spathulenol (16.91%), trimethyl pentaacetane (10.64%), isospathulenol (9.94%) and hexadecanoic acid (6.22%), respectively and 40% VLC fraction of n-hexane extract has the highest terpenoid compounds such as spathulenol (20.4%), caryophyllene oxide (14.25%) and hexadecanoic acid (11.2%). In this regard, many studies have proven the cytotoxic role of compounds in plant essential oils. For example, Oliveira et al. Reported the cytotoxic effects of active volatile oil of several plants with medicinal properties on cancer cell lines such as tumor cell lines murine melanoma (B16F10), human colon carcinoma (HT29), human breast adenocarcinoma (MCF-7), human cervical adenocarcinoma (HeLa), human hepatocellular liver carcinoma (HepG2), human glioblastoma (MO59J, U343, and U251), and Normal hamster lung fibroblasts (V79 cells) were studied. They concluded that compounds such as spathulenol and caryophyllene have significant cytotoxic effects as part of the active compounds present in the plant [42]. In another study by Mellado et al., The effect of essential oils of *Ephedra chilensis* extract on a variety of cancer cell lines was investigated. Using GC-MS analysis, 2.4% of the total compounds in dichloromethane extract are tetradcanoic acid and hexadecanoic acid, which have significant cytotoxic effects on prostate and breast cell lines [43].

Conclusion

The results showed that 40% fraction of n-Hex extract and *F. vulgaris* essential oil on SW-872 cells have a significant cytotoxic effects. The cytotoxicity of the fraction is completely dose-dependent and significantly ($p <0.0001$) higher than the control group. Furthermore, the mechanism of cell death in 40% n-Hex fraction is predominantly through induction of apoptosis, which can be concluded that apoptosis-inducing compounds are probably in the effective fractions of n-Hex extract. It is worth mentioning that the 40% n-Hex fraction has a selective effect on SW-872 cancer cells compared to normal non-cancer cells. The appropriate properties mentioned in the effective samples have raised great hopes for the purification of effective anti-cancer compounds with minimal side effects from *F. vulgaris* in future works.

Declarations

Ethics approval and consent to participate

There is no involvement of human or animal in this study.

Consent for publication

All other authors declare no conflict of interest.

Availability of data and materials

The authors confirm that the data supporting the findings of this study are available within the article.

Competing interests

We declare that we have no significant competing financial, professional, or personal interests that might have influenced the performance or presentation of the work described in this manuscript.

Funding

This work was supported by Drug applied research center, (grant NO: 59209), Tabriz University of Medical Sciences.

Authors' contributions

SJ and KH performed the experiments; PA and VT analyzed of data; KH, VT, AD and PA prepared the manuscript; KH and VT wrote and edited the manuscript; PA designed the experiments; PA led and supervised the project.

Acknowledgements

We would like to thank Dr. Baradaran for his supportive help.

References

1. Organization WH. cancer. December 2018.

2. Mathur P, Sathishkumar K, Chaturvedi M, Das P, Sudarshan KL, Santhappan S, et al. Cancer statistics, 2020: report from national cancer registry programme, India. JCO Global Oncology. 2020;6:1063-75.

3. Saunders FR, Wallace HM. On the natural chemoprevention of cancer. Plant Physiology and Biochemistry. 2010;48(7):621-6.

4. Sithranga Boopathy N, Kathiresan K. Anticancer drugs from marine flora: an overview. Journal of oncology. 2010;2010.

5. Prakash O, Kumar A, Kumar P. Anticancer potential of plants and natural products. Am J Pharmacol Sci. 2013;1(6):104-15.

6. Weli AM, AL-Hinai JR, Al-Mjrafi JM, Alnaaimi JR, Hossain MA, Saeed S, et al. Effect of different polarities leaves crude extracts of Omani juniperus excels on antioxidant, antimicrobial and cytotoxic activities and their biochemical screening. Asian Pacific Journal of Reproduction. 2014;3(3):218-23.

7. Kang JX, Liu J, Wang J, He C, Li FP. The extract of huanglian, a medicinal herb, induces cell growth arrest and apoptosis by upregulation of interferon-β and TNF-α in human breast cancer cells. Carcinogenesis. 2005;26(11):1934-9.

8. Mohammadi A, Mansoori B, Aghapour M, Baradaran PC, Shajari N, Davudian S, et al. The herbal medicine Utrica dioica inhibits proliferation of colorectal cancer cell line by inducing apoptosis and arrest at the G2/M phase. Journal of gastrointestinal cancer. 2016;47(2):187-95.

9. Mohammadi A, Mansoori B, Goldar S, Shanehbandi D, Mohammadnejad L, Baghbani E, et al. Effects of Urtica dioica dichloromethane extract on cell apoptosis and related gene expression in human breast cancer cell line (MDA-MB-468). Cellular and Molecular Biology. 2016;62(2):62-7.

10. Portt L, Norman G, Clapp C, Greenwood M, Greenwood MT. Anti-apoptosis and cell survival: a review. Biochimica et Biophysica Acta (BBA)-Molecular Cell Research. 2011;1813(1):238-59.

11. Sun S-Y, Hail Jr N, Lotan R. Apoptosis as a novel target for cancer chemoprevention. Journal of the national cancer institute. 2004;96(9):662-72.

12. Thangam R, Sathuvan M, Poongodi A, Suresh V, Pazhanichamy K, Sivasubramanian S, et al. Activation of intrinsic apoptotic signaling pathway in cancer cells by Cymbopogon citratus polysaccharide fractions. Carbohydrate polymers. 2014;107:138-50.

13. Felipe KB, Kviecinski MR, da Silva FO, Bücker NF, Farias MS, Castro LSEPW, et al. Inhibition of tumor proliferation associated with cell cycle arrest caused by extract and fraction from Casearia sylvestris (Salicaceae). Journal of ethnopharmacology. 2014;155(3):1492-9.

14. Moshafi MH, Mehrabani M, Mahdikhani S, Saffari F. Antibacterial effects of different fractions of extract of Falcaria vulgaris bernh fruits and bioautography of its effective fraction. Medical Journal of Tabriz University of Medical Sciences and Health Services. 2015;36(6):74-9.

15. Shakibaie D, Pasharavesh L, Khoshboo S, Kaboodi B. The effect of the “Falcaria vulgaris” on deep skin wound remodeling time and skin tension power in rats. J Kermanshah Univ Med Sci. 2007;10(3):187-94.

16. Choobkar N. Effect of using Falcaria vulgaris on skin wound healing and immune response of common carp (Cyprinus carpio). Veterinary Clinical Pathology The Quarterly Scientific Journal. 2015;9(1 (33) Spring):1-9.

17. Yadegari M, Khazaei M, Ghorbani R, Rezae M, Izadi B, Sheikholeslam A. Wound Healing Effect of Falcaria Vulgaris’ Leaves on Aspirin Induced Gastric Ulcer in Rats. Journal of Kermanshah University of

Medical Sciences. 2006;10(3).

18. Gadekar R, Singour P, Chaurasiya P, Pawar R, Patil U. A potential of some medicinal plants as an antiulcer agents. Pharmacognosy reviews. 2010;4(8):136.

19. Khazaei M, Salehi H. PROTECTIVE EFFECT OF OF FALCARIA VULGARIS EXTRACT ON ETHANOL INDUCED GASTRIC ULCER IN RAT. 2006.

20. Yadegari M, Khazaei M, Hamzavi Y, Toloei AR. Antifertility effects of Falcaria vulgaris in female rat. Journal of Arak University of Medical Sciences. 2011;14(2):94-9.

21. Abolhassanzadeh Z, Aflaki E, Yousefi G, Mohagheghzadeh A. Medicinal plants for joint pain in traditional Persian medicine. Trends in Pharmaceutical Sciences. 2016;2(2).

22. Rouhi-Boroujeni H, Asadi-Samani M, Moradi M-T. A review of the medicinal plants effective on headache based on the ethnobotanical documents of Iran. Der Pharmacia Lettre. 2016;8(3):37-42.

23. GOUDINI A, SHAKIBAEI D. EFFECT OF FALCARIA VULGARIS EXTRACT ON ISOLATED RAT HEART. 2007.

24. Jaberian H, Piri K, Nazari J. Phytochemical composition and in vitro antimicrobial and antioxidant activities of some medicinal plants. Food chemistry. 2013;136(1):237-44.

25. KhanAhmadi M, Shahrezaei F. Study on Chemical Constituents of the Essential Oil of Falcaria vulgaris Bernh. Journal of Medicinal plants. 2007;3(23):52-7.

26. Masoudi S, Ameri N, Rustaiyan A, Moradalizadeh M, Azar PA. Volatile constituents of three Umbelliferae herbs: Azilia eryngioedes (Pau) Hedge et Lamond, Laser trilobum (L.) Borkh. and Falcaria falcarioides (Bornm. et Wolff) growing wild in Iran. Journal of Essential Oil Research. 2005;17(1):98-100.

27. Shafaghat A. Volatile oil constituents and antibacterial activity of different parts of Falcaria vulgaris Bernh. growing wild in two localities from Iran. Natural product research. 2011;25(4):368-73.

28. Shafaghat A. Free radical scavenging and antibacterial activities, and GC/MS analysis of essential oils from different parts of Falcaria vulgaris from two regions. Natural product communications. 2010;5(6):1934578X1000500636.

29. Assadi M, Ramak Maassoumi A, Khatamsaz M. Flora of Iran: Ministry of Agriculture, Islamic Republic of Iran Tehran; 1989.

30. Wesołowska A, Jadczak P, Kulpa D, Przewodowski W. Gas chromatography-mass spectrometry (GC-MS) analysis of essential oils from AgNPs and AuNPs elicited Lavandula angustifolia in vitro cultures. Molecules. 2019;24(3):606.

31. WHO. cancer. Sep 2018.

32. Hemalswarya S, Doble M. Potential synergism of natural products in the treatment of cancer. Phytotherapy Research: An International Journal Devoted to Pharmacological and Toxicological Evaluation of Natural Product Derivatives. 2006;20(4):239-49.

33. Johnstone RW, Ruefli AA, Smyth MJ. Multiple physiological functions for multidrug transporter P-glycoprotein? Trends in biochemical sciences. 2000;25(1):1-6.

34. Xu G, Shi Y. Apoptosis signaling pathways and lymphocyte homeostasis. Cell research. 2007;17(9):759-71.

35. Pal SK, Shukla Y. Herbal medicine: current status and the future. Asian pacific journal of cancer prevention. 2003;4(4):281-8.

36. Najda A, Dyduch J, Świca K, Kapłan M, Papliński R, Sachadyn-Król M, et al. Identification and Profile of Furanocoumarins from the Ribbed Celery (Apium Graveolens L Var. Dulce Mill./Pers.). Food Science and Technology Research. 2015;21(1):67-75.

37. Shokoohinia Y, Sajjadi S-E, Gholamzadeh S, Fattahi A, Behbahani M. Antiviral and cytotoxic evaluation of coumarins from Prangos ferulacea. Pharmaceutical biology. 2014;52(12):1543-9.

38. Orhan IE, Senol FS, Shekfeh S, Skalicka-Wozniak K, Banoglu E. Pteryxin-A promising butyrylcholinesterase-inhibiting coumarin derivative from Mutellina purpurea. Food and Chemical Toxicology. 2017;109:970-4.

39. Beltagy AM, Beltagy DM. Chemical composition of Ammi visnaga L. and new cytotoxic activity of its constituents khellin and visnagin. Journal of Pharmaceutical Sciences and Research. 2015;7(6):285.

40. Jun NJ, Mosaddik A, Moon JY, Ki-Chang J, Dong-Sun L, Ahn KS, et al. Cytotoxic activity of [beta]-Caryophyllene oxide isolated from jeju guava (Psidium cattleianum sabine) leaf. Records of Natural Products. 2011;5(3):242.

41. Martins A, Hajdú Z, Vasas A, Csupor-Löffler B, Molnár J, Hohmann J. Spathulenol inhibit the human ABCB1 efflux pump. Planta Medica. 2010;76(12):P608.

42. de Oliveira PF, Alves JM, Damasceno JL, Oliveira RAM, Dias HJ, Crotti AEM, et al. Cytotoxicity screening of essential oils in cancer cell lines. Revista Brasileira de Farmacognosia. 2015;25(2):183-8.

43. Mellado M, Soto M, Madrid A, Montenegro I, Jara-Gutiérrez C, Villena J, et al. In vitro antioxidant and antiproliferative effect of the extracts of Ephedra chilensis K Presl aerial parts. BMC complementary and alternative medicine. 2019;19(1):1-10.

Tables

Table 1. Weighing results of extracts obtained from *F. vulgaris* and its fractionation of n-Hex and DCM extracts.

MeOH		DCM		n-Hex		Extract
9.04		2.12		1.35		Weight of plant extract (g/100 g of extract)
100%	80%	60%	40%	20%	10%	Fractions
4.35	16.25	8.6	12.35	20.05	4.55	Weight of n-Hex extract fraction (g/100 g of extract)
11.95	13.15	10.20	2.25	1.30	0	Weight of DCM extract fraction (g/100 g of extract)

Table 2. Cytotoxic effect of n-Hex, DCM and MeOH extracts and fractions of n-Hex extract of *F. vulgaris* on SW-872 cells

(SW-872 cells) IC_{50}(µg/ml)		Sample	
48 hours	24 hours		
108.70 ± 5.72	138.40 ± 3.89	n-Hex extract	
163.70 ± 6.92	209 ± 24.53	DCM extract	
> 800	> 800	MeOH extract	
> 300	> 300	10% Fraction	n-Hex extract fractions
68.16 ± 3.17	74.22 ± 5.73	20% Fraction	
24.42 ± 0.09	32.04 ± 1.27	40% Fraction	
48.92 ± 13.95	48.13 ± 2.73	60% Fraction	
63.34 ± 0.01	85.36 ± 3.54	80% Fraction	
146.40 ± 12.02	168.40 ± 31.75	100% Fraction	

Table 3. Cytotoxic effect of n-Hex, DCM and MeOH extracts and fractions of n-Hex extract of *F. vulgaris* on HFFF cells

HFFF cells) (IC_{50}(µg/ml)		Sample	
48 hours	24 hours		
46.11 ± 8.41	122.70 ± 19.58	n-Hex extract	
60.68 ± 10.34	76.26 ± 16.38	DCM extract	
> 800	> 800	MeOH extract	
> 300	> 300	10% Fraction	n-Hex extract fractions
47.54 ± 3.73	40.95 ± 1.36	20% Fraction	
33.84 ± 0.46	49.30 ± 0.49	40% Fraction	
29.55 ± 0.59	11.38 ± 2.34	60% Fraction	
49.27 ± 1.49	55.26 ± 2.67	80% Fraction	
48.38 ± 5.47	114.10 ± 8.34	100% Fraction	

Table 4. Specifications of volatile compounds identified in essential oils using GC-MS

No	Name	Rt	Area%	KI
1	Octanal	18.62	3.93	981
2	o-Cymene	20.26	0.6	1013
3	2-Nonanone	23.44	0.58	1072
4	Nonanal	24.06	0.64	1084
5	2-Decenal	32.11	2.48	1240
6	Copaen	38.83	1.45	1380
7	trans-Caryophyllene	40.78	3.57	1422
8	beta.-copaen-4 .alpha.-ol	42.26	1.36	1455
9	Naphthalene	43.10	2.56	1474
10	germacrene d	43.37	1.29	1480
11	Delta-Cadinene	45.02	4.49	1519
12	caryophyllene oxide	46.14	18.3	1545
13	1,5-epoxysalvial-4(14)-ene	46.81	2.63	1561
14	Isospathuleno	47.08	4.31	1567
15	(+) spathulenol	47.30	33.8	1573
16	Salival-4(14)-en-1-one	47.82	3.23	1585
17	Epiglobulol	48.16	1.85	1593
18	Ledenoxide-(I)	48.64	1.19	1605
19	Aromadendrene epoxide-(II)	49.40	4.84	1625
20	Calarenepoxide	51.36	3.89	1674
21	Kauran-18-al	54.28	1.51	1751
	Identified		**98.23**	
	Non terpenoids		**11.7**	
	terpenoids		**86.53**	
	Monoterpene hydrocarbones		**0.6**	
	sesquiterpene hydrocarbones		**10.8**	
	oxygenated sesquiterpene		**75.3**	

Table 5. Characterization of volatile compounds identified in n-Hex extract.

No	Name	Rt	Area%	KI
1	Hexene	5.062	5.72	585
2	Octanoic Acid	16.688	3.31	1157
3	Dodecane	18.031	1.08	1200
4	Tetradecane	23.923	17.64	1401
5	isospathulenol	28.436	9.94	1571
6	(-)-Spathulenol	28.652	16.91	1580
7	SALVIAL-4(14)-EN-1-ONE	29.017	1.28	1594
8	2,6,10-Trimethylpentadecane	29.183	10.64	1600
9	Widdrol	30.267	1.66	1645
10	Himbaccol	30.526	1.18	1656
11	1-[1-Methoxy-3,3-dimethyl-2-(3-methylbuta-1,3-dienyl)cyclopentyl]ethanone	31.356	1.18	1690
12	Limonene dioxide	31.581	3.09	1700
13	9,10Dimethyltricyclo [4.2.1.1(2,5)] decane-9, 10-diol	32.18	1.16	1725
14	Isoaromadendrene epoxide	33.122	3.62	1766
15	Octadecane	33.896	3	1800
16	2-Butenal, 2-methyl-4-(2,6,6-trimethyl-1-cyclohexen-1-yl)-	34.397	4.76	1823
17	Hexahydrofarnesyl acetone	34.61	1.16	1833
18	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	34.741	1.14	1839
19	Hexadecanoic acid	36.997	6.22	1944
20	Hexadecanoic acid, ethyl ester	37.695	2.16	1978
	Identified		**96.85**	
	Non-terpenoids		**48.97**	
	terpenoid		**47.88**	
	oxygenated monoterpene		**3.09**	
	oxygenated sesquiterpene		**29.69**	
	oxygenated diterpene		**1.16**	

Table 6. Specifications of compounds identified in 40% VLC fraction of n-Hex extract.

No	Name	Rt	Area%	KI
1	Octanoic Acid	16.27	3.31	1157
2	trans-Caryophyllene	18.30	2.30	1422
3	germacrene d	20.40	2.15	1480
4	caryophyllene oxide	23.20	14.25	1545
5	(-)-Spathulenol	27.68	20.4	1580
6	Widdrol	30.67	6.70	1645
7	Limonene dioxide	37.87	4.20	1700
8	Octadecane	42.61	5	1800
9	Hexadecanoic acid	46.52	11.20	1944
10	Hexadecanoic acid, ethyl ester	47.60	1.12	1978
	Identified		**70.47**	
	Non-terpenoids		**22.72**	
	terpenoid		**47.75**	
	monoterpene		**4.20**	
	sesquiterpene		**43.55**	

Figures

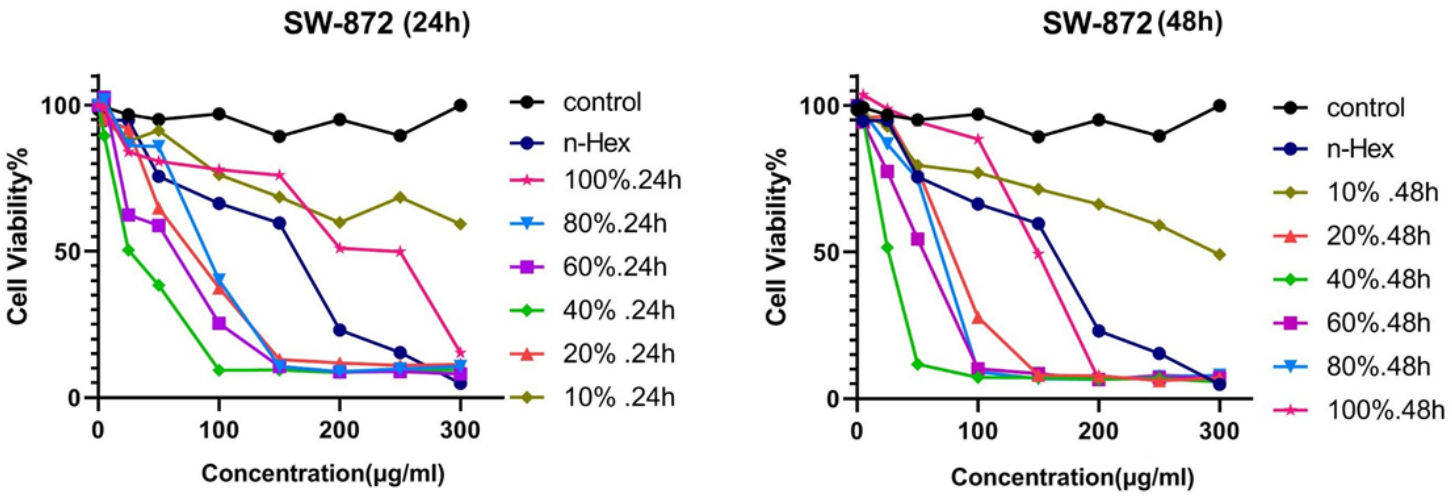


Figure 1

Determination of viability of SW-872 cells treated with different concentrations of n-Hex extract fractions of F. vulgaris using MTT method at 24 hours and 48 hours.

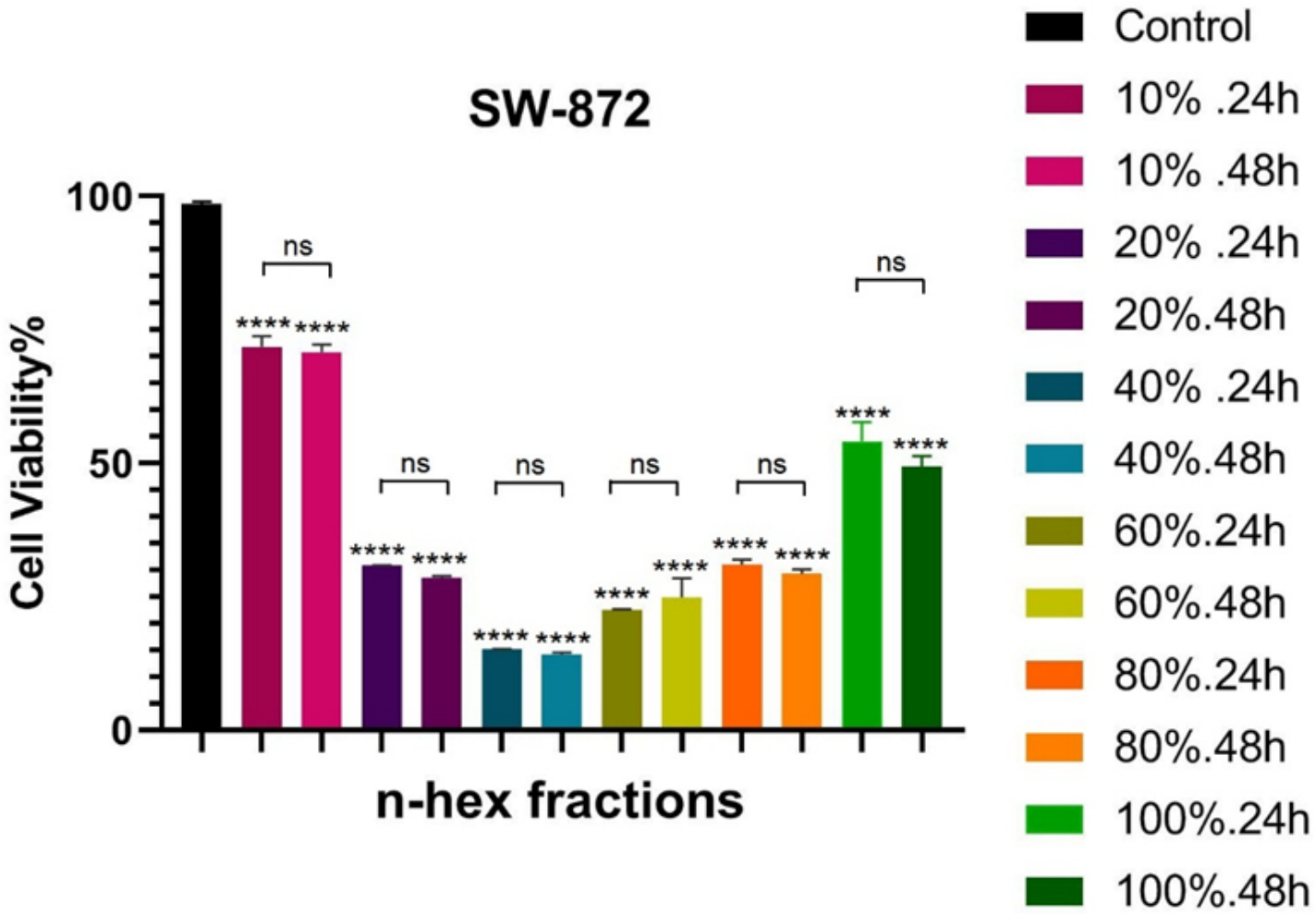
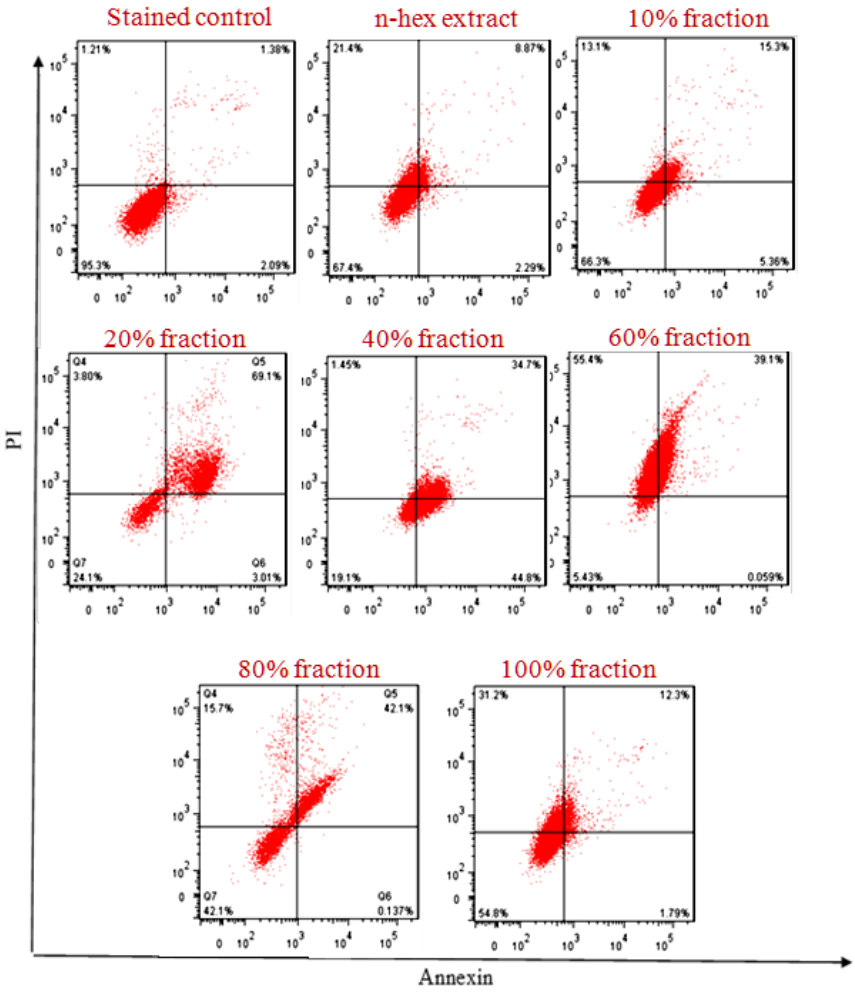
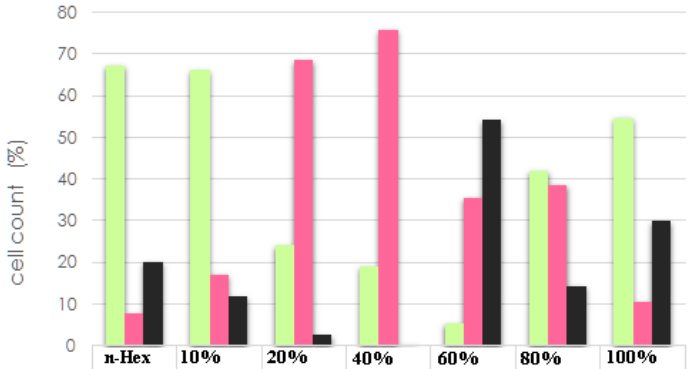


Figure 2

Statistical comparison of viability of SW-872 cells treated with n-Hex extract of F. vulgaris compared to the control group, using MTT method.





	n-Hex extract	10% Fraction	20% Fraction	40% Fraction	60% Fraction	80% Fraction	100% Fraction
Live Cells	67.4	66.3	24.1	19.1	5.43	42.1	54.8
Apoptosis	7.69	17.19	68.64	76.03	35.689	38.767	10.62
Necrosis	20.19	11.89	2.59	0.24	54.19	14.49	29.99

Figure 3

a) Apoptosis rate of cells treated with n-Hex extract fractions on SW-872 cells. b) Comparison of the mechanism of SW-872-induced cell death with n-Hex extract and its fractions.