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CYTOTOXIC ACTIVITY OF *CROCUS PALLASII* SUBSP. *HAUSSKNECHTII* IN HUMAN CANCER CELL LINES

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ABSTRACT: *Crocus pallasii* subsp. *haussknechtii* grows in the west of Iran where local people consume the corm. It belongs to a saffron family (Iridaceae), which has shown several biological activities including cytotoxic and anti-angiogenesis properties. The corm (the nut and the surrounding fibers) and aerial parts of the species have shown anti-angiogenesis properties in HUV-EC-C cells previously; in the present study, the cytotoxic activity of different parts of the plant was evaluated. As people consumed the nuts, the aerial parts, nuts, and fibers were dried and ground and extracted separately. The methanol extract and petroleum ether, chloroform and methanol fractions were prepared by maceration and evaluated in A-549, HepG-2, MCF-7 and HT-29 cancer cell lines through MTT assay. The aerial parts showed no toxicity to any of the cancer cell lines up to the concentration of 100 µg/mL. The methanol extract and fraction of the fibers showed cytotoxicity to all cell lines while the methanol extract and fraction of the nuts and the chloroform fraction showed cytotoxicity to HepG-2 and A-549 cells, respectively.

INTRODUCTION: The genus *Crocus* (Iridaceae) comprises several species growing in the Mediterranean region and Iran. These plants usually need a four seasoned climate with the growing phase during autumn to spring and the corm being protected from summer hotness under the ground. In most species, the aerial parts start to grow by starting the autumn rain and grow flowers afterward. Some develop leaves and flowers at the same time while in others the flowers bloom after the leaves have appeared.

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There are some species which blossom in the spring when the weather is a bit warmer. Nine species of *Crocus* grow in Iran one of which is the popular *Crocus sativus* (saffron) which is used in culinary as a flavoring and coloring agent worldwide [1, 2].

Crocus sativus L. is cultivated in the North-Eastern part of the country. Another species of this genus is *Crocus pallasii* subsp. *haussknechtii* (Boiss. & Reut. ex Maw) B. Mathew growing in the western regions of Iran, blooming during October-November [3]. The plant owns a corm covered with fibers; the inner nut is usually consumed by local people. Unlike saffron which is found to possess antioxidant, anti-angiogenesis, and cytotoxic properties [4, 5, 6, 7], there is little known about the biological activities of *C. pallasii* subsp. *haussknechtii*.

These studies include the anticonvulsant activity of the corms hydroalcoholic extract in male bulb c mice [8] and the anti-angiogenesis property of the corm methanol extract in a wound healing assay [9]. It should be noted that in the above *in-vivo* study, the plant did not exhibit the activity in non-toxic doses; considering this result and the reports about the cytotoxicity of *Crocus sativus* (another close species of this genus) and the previously reported anti-angiogenesis activity [9], the present study was designed to evaluate the cytotoxicity of the plant to cancer cell lines.

MATERIAL AND METHODS:

Plant Material: *Crocus pallasii* subsp. *haussknechtii* whole plant was collected from Kermanshah province, Iran during spring 2015. The plant was authenticated at the Herbarium of Traditional Medicine and Materia Medica Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran. A voucher specimen was provided for future reference (TMRC-3604).

Preparation and Extraction: The aerial parts, the nut and the fiber cover of the corm were separated and dried individually in the shade. 10 g of each sample was macerated with 100 mL methanol for 24 h with continuous shaking. The method was repeated three times; each time the mixtures were filtered and the fresh solvent was used. The filtrates of each sample were combined, concentrated (using a rotary evaporator), dried and kept in the refrigerator for further assays. Twenty g of the aerial parts, the nut and the fiber cover of the corm were again macerated this time with 200 mL petroleum ether, chloroform and methanol successively with the same above method to prepare the fractions.

MTT Assay: Human lung carcinoma (A-549), human hepatocellular carcinoma (HepG-2) human colorectal carcinoma (HT-29) and human breast adenocarcinoma (MCF-7) cell lines were provided from Pasture Institute, Iran. The cells were seeded in 96-plates (8500, 10000, 5000 and 9000 cells for A-549, HepG-2, HT-29 and MCF-7 cell lines, respectively). The extracts and fractions were dissolved in DMSO to prepare the samples for the MTT assay. The final concentration of DMSO was 1%. Twenty-four hours later, the cells were exposed to different concentrations of extract/ fractions for 72 h. By the end of the exposure, the cells were washed with PBS (phosphate buffered saline), and 100 µL MTT solution (final concentration of 0.05 mg/mL) was added to each well. The absorbance was recorded four h later with an ELISA reader at 570 nm after adding DMSO to the wells for dissolving the formed formazan crystals; 5-FU was used as the positive control. The viability of the cells was calculated using the following formula. The experiments were performed in triplicate for the nine samples (three extracts and six fractions, the petroleum ether fractions were excluded from the study because they did not dissolve in DMSO).

$$\text{Viability} = (A_s/A_d) \times 100$$

Where, A_s is the absorbance of the sample and A_d is the absorbance of DMSO 1% as the negative control. IC_{50} was calculated by plotting the viability of the cells vs. concentrations with Microsoft Excel program [10, 11, 12, 13, 14].

RESULTS AND DISCUSSION: An initial step for assessing cytotoxicity could be fulfilled using the MTT method. In this simple colorimetric assay, the cells would be exposed to the materials to tested for a specified duration. The material would be removed in the next step followed by confronting the MTT solution which persuades the dehydrogenase enzymes in the live cells to convert the yellow 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (known as MTT) to purple formazan crystals. The absorbance of these crystals dissolved in a suitable solvent such as DMSO would be correlated to the number of live cells. The results obtained by MTT assay in the four cancer cell lines have been presented in **Table 1** and **Fig. 1 - 4**.

TABLE 1: CYTOTOXIC ACTIVITY OF *CROCUS PALLASII* SUBSP. *HAUSSKNECHTII* IN CELL LINES

	IC_{50} (µg/mL)			
Sample	**A-549**	**HePG-2**	**HT-29**	**MCF-7**
FEx	30.6	45.3	14.7	42.6
FCl	38.3	-	-	-
FMe	52.8	33.4	15.9	21.8
AEx	-	-	-	-
ACl	-	-	-	-
AMe	-	-	-	-
CEx	97.8	68.0	-	-
CCl	76.8	-	-	-
CMe	35.0	69.5	-	-
5-Fu	3.1	0.4	3.9	13.9

F: fiber; A: aerial parts; C: corm nut; Ex: Methanol extract; Cl: chloroform fraction; Me: methanol fraction; -: No toxicity was observed.

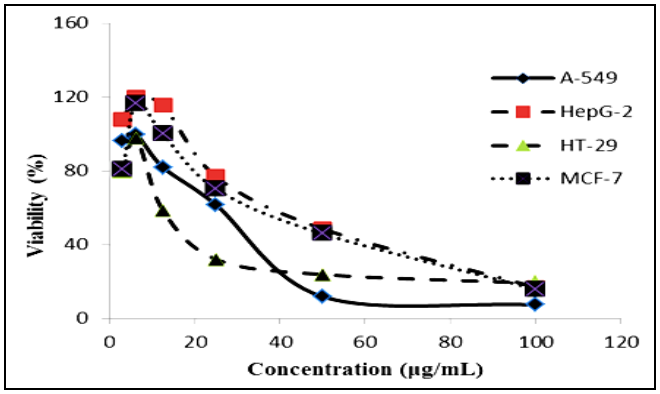

FIG. 1: CYTOTOXICITY OF CORM COVERING FIBERS METHANOL EXTRACT IN A-549, HepG-2, HT-29 AND MCF-7 CELLS

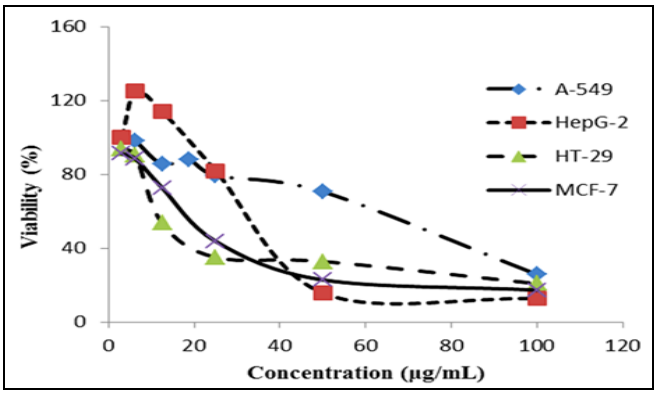

FIG. 2: CYTOTOXICITY OF CORM COVERING FIBERS METHANOL FRACTION IN A-549, HepG-2, HT-29 AND MCF-7 CELLS

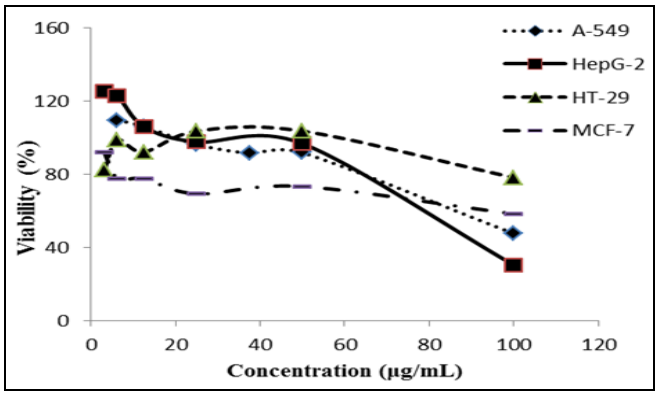

FIG. 3: CYTOTOXICITY OF CORM NUT METHANOL EXTRACT IN A-549, HepG-2, HT-29 AND MCF-7 CELLS

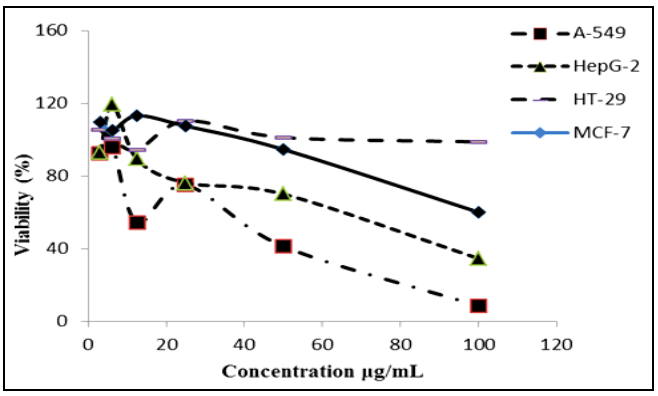

FIG. 4: CYTOTOXICITY OF CORM NUT METHANOL EXTRACT IN A-549, HepG-2, HT-29 AND MCF-7 CELLS

Neither the methanol extract nor the fractions of the aerial parts showed toxicity to any of the cell lines; while the methanol extract and fraction of the fiber were cytotoxic to all cell lines. Also, the extract and methanol fraction of the corm nut demonstrated toxicity only to the hepatocellular and lung carcinomas. For chloroform fraction, the effect was admitted in A-549 cells for both the fibers and the nuts. The lowest IC_{50} was found for the methanol fraction of the corm fibers in HT-29 cells (15.9 µg/mL).

In a previous study for anti-angiogenesis properties of *C. pallasii* subsp. haussknechti, Mosaddegh *et al.*, had reported the cytotoxicity of the plant against human umbilical vein cells (HUV-EC-C) [9]. They had found that the methanol extract and fractions of the corm (nut and the fiber were not separated) were toxic to the cells with IC_{50} values less than 75 µg/mL. They had reported that the biological activity of the corm was more considerable compared to the aerial parts. They could not define whether the observed effects were due to the fibers or the edible parts.

This is in agreement for the present study that only the corm (both the nuts and the fibers) showed effects in tested cell lines. The most considerable effects were found for the more polar portions suggesting that compounds of higher polarities would be responsible for the observed biological activity. This is more prominent for the corm fibers where all cell lines showed sensitivity for the methanol extract and fraction. It would be of value to isolate the components of the corm (especially the fiber) to continue the cytotoxicity studies. Tumor growth and invasion are very much indebted to angiogenesis since cancer cells need to grow vessels to develop and spread through the body [15]. One way to inhibit tumors is to suppress the pro-angiogenesis by anti-angiogenesis agents [16]. The *in -vitro* anti-angiogenesis properties of the corm [9] and the cytotoxicity observed in tumor cell lines in the present study, may suggest *C. pallasii* subsp. haussknechti as a suitable candidate for further cancer studies.

Considering the cytotoxicity of the nut methanol extract and fraction to the A-549 and HepG-2 cells,

it comes to the mind that the edible part might also be cytotoxic to humans. As mentioned earlier, the nuts have been used freely by local people for many years; so, two points come to the mind. First is that the nuts may be toxic and harmful for human health and local people should be warned about the probable unwanted effects; the second is considering that our experiment was an *in-vitro* examination and that the toxic content of the nuts may decompose in the body and lose their toxicity.

Also, people use the nut freshly in spring, so the amount and time of consumption are nearly limited and although toxic signs may not find time to appear, they may still exist and could be harmful and trigger cell toxicity.

CONCLUSION: Regarding that safety is of great importance in consumption of herbal materials and considering the results of the present study; the hypotheses as mentioned earlier must be further evaluated to confirm the safety or harmfulness of the edible parts of *C. pallasii* subsp. *haussknechti*.

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DECLARATION OF INTEREST: The authors declare that there is no conflict of interest.

REFERENCES:

1. Mozaffarian V: A Dictionary of Plant Names. Farhange-e-Moaser Publishers, Edition 1st, 1999.
2. Erol O, Betül Kaya H, Şik L, Tuna M, Can L and Tanyolaç MB: The genus *Crocus*, series *Crocus* (Iridaceae) in Turkey and 2 East Aegean islands: a genetic approach. Turkish Journal of Biology 2014; 38: 48-62.
3. Mazhari N: Flora of Iran: Iridaceae. Research Institute of Forests and Rangelands Publishers 1999.
4. Srivastava R, Ahmed H, Dixit RK, Dharamveer and Saraf SA: *Crocus sativus* L. A comprehensive review. Pharmacognosy Reviews 2010; 4: 200-208.
5. Mousavi SH, Moallem SA, Mehri S, Shahsavand S, Nassirli H and Malaekeh-Nikouei B: Improvement of cytotoxic and apoptogenic properties of crocin in cancer cell lines by its nanoliposomal form. Pharmaceutical Biology 2011; 49: 1039-1045.
6. Umigai N, Tanaka J, Tsuruma K, Shimazawa M and Hara H: Crocetin, a carotenoid derivative, inhibits VEGF-induced angiogenesis *via* suppression of p38 phosphorylation. Current Neurovascular Research 2012; 9: 102-109.
7. Samarghandian S, Tavakkol Afshari J and Davoodi S: Suppression of pulmonary tumor promotion and induction of apoptosis by *Crocus sativus* L. extraction. Applied Biochemistry and Biotechnology 2011; 164: 238-247.
8. Sayyah M, Faraji H, Dehghani A, Bakhtiari H, Kamalinejad M and Narenjkar J: Screening of the anticonvulsant potential of some common medicinal plants of Iran in pentylenetetrazole and maximal electroshock seizure models in male mice. Physiology and Pharmacology 2011; 15: 66-71
9. Mosaddegh, Esmaeili S, Eslami-Tehrani B, Kermatian B, Mohebby S and Hamzeloo-Moghadam M: Anti-angiogenesis properties of *Crocus pallasii* subsp. *haussknechtii*, a popular ethnic food. Research Journal of Pharmacognosy 2015; 2: 37-42.
10. Mosaddegh M, Taheri M, Eslami Tehrani B and Hamzeloo-Moghadam M: Apoptotic potential of two Caryophyllaceae species in MCF-7 and MDA-MB-468 cell lines. Research Journal of Pharmacognosy 2018; 5: 63-69
11. Fallahian F, Aghaei M, Abdolmohammadi MH and Hamzeloo-Moghadam M: Molecular mechanism of apoptosis induction by Gaillardin, a sesquiterpene lactone, in breast cancer cell lines Gaillardin-induced apoptosis in breast cancer cell lines. Cell Biology and Toxicology 2015; 31: 295-305
12. Hamzeloo-Moghadam M, Aghaei M, Fallahian F, Jafari SM, Dolati M and Abdolmohammadi MH: Britannin, a sesquiterpene lactone, inhibits proliferation and induces apoptosis through the mitochondrial signaling pathway in human breast cancer cells. Tumor Biology 2016; 36: 1191-1198.
13. Hasani Sadrabadi Z, Majd A, Hamzeloo-Moghadam M and Irian S: Effect of the electromagnetic field on okra (*Hibiscus esculentus* L.) developmental stages and the effect of okra extract on breast cancer cells Research Journal of Pharmacognosy 2016; 3: 41-45.
14. Hajimehdipoor H, Gharanjik B, Soleimani H, Mousavi Z and Hamzeloo-Moghadam M: Two algae from Oman sea: the *in-vitro* biological effects. International Journal of Pharmaceutical Sciences and Research 2017; 8: 4286-4290.
15. Lee MS, Moon EJ, Lee SW, Kim MS, Kim KW and Kim YJ: Angiogenic activity of pyruvic acid in *in-vivo* and *in-vitro* angiogenesis models. Cancer Research 2011; 61: 3290-3293.
16. Bian W, Chen F, Bai L, Zhang P and Qin W: Dihydrotanshinone I inhibits angiogenesis both *in-vitro* and *in-vivo*. Acta Biochimica et Biophysica Sinica 2008; 40: 1-6.

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