

Peer Review File

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Reviewer A

Comment 1: Spelling should be checked, some writing mistakes.

Reply 1: In the revised manuscript, the spelling and writing mistakes have been corrected.

Changes in the text: The title “Effects of saffron and its active constituent crocin on cancer chemoprevention” has been changed to “Effects of saffron and its active constituent crocin on cancer management”. For other changes, please see responses below. In the Future Perspective section, “chemtherapy” and “inlues” have been corrected to “chemotherapy” and “includes”, respectively.

Comment 2: Abbreviations should be explained for example line 139.

Reply 2: On line 139, the abbreviation “BP” has been replaced by its full word “benzo[a]pyrene”.

Changes in the text: “BP” has been changed to “benzo[a]pyrene”.

Comment 3: Line 70 and following: Should be formulated more generally, not limited to ginseng (including relevant literature).

Reply 3: The phrase, “Certain herbs” has been replaced with the more general wording, “herbal medicines”, and references have been updated.

Changes in the text: “certain herbs, such as Panax ginseng” has been changed to “herbal medicines”, and new references have been added:

13. Chung VC, Wu X, Hui EP, et al. Effectiveness of Chinese herbal medicine for cancer palliative care: overview of systematic reviews with meta-analyses. *Sci Rep* 2015;5:18111.
14. Sagbo IJ, Otang-Mbeng W. Plants used for the traditional management of cancer in the eastern cape province of south africa: a review of ethnobotanical surveys, ethnopharmacological studies and active phytochemicals. *Molecules* 2021;26.

Comment 4: Line 83: Repetitions should be avoided (see line 78). Furthermore, molecular structure of the components listed in the review-article should be shown in a figure.

Reply 4: To avoid repetitions, “Iran” has been removed. In addition, based on the comments from the editor, Figure 1 has been deleted.

Changes in the text: “grows in some Mediterranean countries, Iran, India, regions in China such as Tibet” has been changed to “grows in some Mediterranean countries, India, and some regions in China, such as Tibet.”

Comment 5: Line 131: “Inhibitory effects” should be explained in more detail. Which proteins and/or biological behaviour have been inhibited? The same with lines 133-137.

Reply 5: In this sentence, “inhibitory effects” refers to the cancer cell growth inhibition. We have modified the expression, including the lines of 133-137.

Changes in the text: “concentration-dependent inhibitory effects of saffron on cancer cells were observed.” has been changed to “concentration-dependent cancer cell growth inhibitory effects of saffron were observed.”; “significant inhibitory effects on three human cancer cell lines” has been changed to “significant cell growth inhibitory effects on three human cancer cell lines”.

Comment 6: Which parameters have been altered by dimethyl-crocin, crocin, and crocin?

Reply 6: Based on the literature, the IC₅₀ for dimethyl-crocin, crocin, and crocin are 0.8, 2 and 2 μM, respectively. To clearly present the activities of those compounds, in the revised manuscript, we have modified the statement.

Changes in the text: In the text, “with low ID₅₀ (less than 2 μM)” has been modified to “with low ID₅₀, which are less than 2 μM”.

Comment 7: Line 147: I would suggest that Crocus extracts rather than Crocus species may inhibit cell growth.

Reply 7: Thank you. The text has been changed according to your comments.

Changes in the text: “Crocus species” has been changed to “Crocus extracts”

Comment 8: Line 151: Saffron blocks growth of fibroblasts which might contradict the statements given before (no effect on normal, healthy cells). This should be explained.

Reply 8: To explain the different effects of saffron on different types of cells, the expression has been modified in the revised manuscript.

Changes in the text: “In another study, compared to normal cells, saffron showed much stronger effects on fetal lung fibroblasts cells including A-549, WI-38 and VA-13 cell lines.” has been changed to “In another study, compared to normal lung fibroblasts cells (WI-38), saffron showed much stronger effects on lung cancer cells, including A-549 and VA-13 cell lines.”

Comment 9: Line 165: Reference should be given.

Reply 9: The reference has been added.

Changes in the text: The following reference has been added:

22. Aung HH, Wang CZ, Ni M, et al. Crocin from Crocus sativus possesses significant anti-proliferation effects on human colorectal cancer cells. *Exp Oncol* 2007;29:175-80.

Comment 10: Line 223: This requires further discussion. What’s about dose-dependent effects of saffron?

Reply 10: Dose-dependent effects of saffron on colon cancer have been presented in other, different studies. This study focuses more on the MACC1-dependent effects of saffron. To avoid confusion, in this part, we removed the phrase, “dose-dependent”.

Changes in the text: “In a dose- and MACC1-dependent manner, saffron inhibited colon cancer cell growth and migration of MACC1-expressing cells.” has been changed to “In an MACC1-dependent manner, saffron inhibited colon cancer cell growth and migration.”

Comment 11: Line 316: This is not correct. Mirzaei et al. Efficacy and safety of Jollab (a saffron-based beverage) on cancer-related fatigue in breast cancer patients: A double-blind randomized clinical trial. Complement Med Res. 2022 Jun 29. doi: 10.1159/000525775 . There are also several articles dealing with the quality of life, anxiety and depression, which are all symptoms of cancer patients. It might be important to take care on these articles.

Reply 11: Thank you for pointing out this mistake. In the revised manuscript, this error has been corrected. In addition, recently published clinical studies of saffron/crocin on cancer patients’ quality of life, including doi: 10.1159/000525775, have been added.

Changes in the text: “Cancer management with chemoprevention and chemotherapy, which includes chemically intervention with naturally originated and synthetic drugs, is a commonly used method for fighting against different cancers.” has been changed to “Cancer chemotherapy,

the chemical intervention in cancer with naturally originated and synthetic drugs, is a commonly used treatment used for fighting against different cancers.”

In addition, the following information has been added: “Further clinical studies have been conducted to investigate the effects of saffron on the quality of life in patients with malignancies. A saffron-based beverage showed significant positive effects on reducing fatigue in patients with breast cancer (79). Data from another recent clinical trial indicated that using crocin during chemotherapy in breast cancer patients ameliorated their anxiety and depression (80).”

References:

79. Mirzaei H, Gharehgozlou R, Heydarirad G, et al. Efficacy and safety of Jollab (a saffron-based beverage) on cancer-related fatigue in breast cancer patients: A double-blind randomized clinical trial. *Complement Med Res* 2022.
80. Salek R, Dehghani M, Mohajeri SA, et al. Amelioration of anxiety, depression, and chemotherapy related toxicity after crocin administration during chemotherapy of breast cancer: A double blind, randomized clinical trial. *Phytother Res* 2021;35:5143-53.

Reviewer B

Comment 1: *General.*

The term “Chemoprevention” (or chemopreventive) is not correctly used. Most of the citations throughout the manuscript refer to anticancer effects of the products (in vitro), via the treatment of cultured cancer cells (antiproliferative activity). In other cases, the authors refer to the treatment of cancer-associated symptoms. But there is no chemoprevention. In fact, in Human chemoprevention is almost impossible to demonstrate (hard to demonstrate that a cancer will not appear). I would strongly suggest to revise completely the title and the manuscript to avoid the term “cancer chemoprevention” totally.

Reply 1: Thank you for your important comments. To accurately express the topic of this review, in the revised manuscript, the title has been updated by removing “cancer chemoprevention”.

Changes in the text: The original title “Effects of saffron and its active constituent crocin on cancer chemoprevention” has been modified to “Effects of saffron and its active constituent crocin on cancer management: A narrative review”.

Comment 2: *Cancer cell growth inhibitory effects (line 122), Antitumor effects in animal models (line 156), etc. This is not cancer chemoprevention but simply cancer treatment.*

Reply 2: We agree with the reviewer's opinions that cancer cell growth inhibition and antitumor effects are chemotherapy, but not chemoprevention. In the revised manuscript, "cancer chemoprevention" has been removed from the title, the Abstract, and Keywords.

Changes in the text: The original title, "Effects of saffron and its active constituent crocin on cancer chemoprevention" has been modified to "Effects of saffron and its active constituent crocin on cancer management". In the Abstract, "Then, the effects of saffron and crocin on cancer chemoprevention for different human cancers are presented." has been changed to "Then, we present the anticancer effects of saffron and crocin on different human cancer cells." In Keywords, "chemoprevention" has been removed.

Comment 3: *Data.*

The work reports the weak antiproliferative action of a saffron extract and crocin against two cancer cell lines in vitro. This is extremely limited for a scientific article. There are better anticancer data about crocin in the literature (not cited here: PMID 35739971, 35563808, 35447530, and many more). A table should be included to summarize the anticancer activities (with IC50 values) reported with crocin and different cancer cell types. There is also a need for a Figure that recapitulates the signaling pathways activated by the product. There is a lack of quantitative information. Activity in vivo is cited, but no value given and no comparison with established drugs.

Reply 3: The published literature reported moderate effects of saffron and crocin on different cancer cell lines. However, since there are serious side effects of currently commonly used cancer chemotherapeutic agents, considering the safety of saffron and crocin, moderate anticancer effects are acceptable. That is the reason that more related research has been continuously published recently, including your listed three articles, which were published in 2022. Therefore, we believe this review is necessary, which will attract the reader's attention.

We agree with the reviewer's comments that the IC50 is important data for antiproliferation. However, since IC50s were not reported in many articles, and typically the trend for antiproliferation alone was compiled in bar graphs and presented in those articles, using a table to list IC50 is not compatible with our article, especially since their experimental conditions were

different. Nevertheless, in the revised manuscript, new references have been added, including the several reviewer-listed references, and the author-reported IC50s have been presented.

The anticancer pathways included in the effects of saffron and crocin are important. From what has been seen in available literature, not all articles presented the pathways. Even though some reports explored anticancer pathways, most of them were not well verified. Since there are no established pathways for saffron and crocin, we did not draft a graph to illustrate the pathways; instead, the reported anticancer pathways have been presented in the main text.

Changes in the text: In the text, “Researchers also reported the inhibitory effects of dimethylcrocetin, crocetin, and crocin on human K562 chronic myelogenous leukemia cells and HL-60 promyelocytic leukemia cells with low ID₅₀ with low ID₅₀ (less than 2 μM)” has been modified to “Researchers also reported the inhibitory effects of dimethylcrocetin, crocetin, and crocin on human K562 chronic myelogenous leukemia cells and HL-60 promyelocytic leukemia cells with low ID₅₀, which are less than 2 μM”.

In the revised manuscript, the following has been added: “Recently, saffron and crocins were found to have antiproliferative effects on glioblastoma and rhabdomyosarcoma cells (34).” and, “Recent studies demonstrated that crocin inhibits angiogenesis and colorectal cancer cell metastasis by targeting NF-κB and blocking TNF-α/NF-κB/VEGF pathways (74). Moreover, crocin inhibited NF-κB-mediated inflammation and proliferation in cancer cells through down-regulating protein levels of protein kinase C theta (75).”

References:

34. Hatziagapiou K, Nikola O, Marka S, et al. An in vitro study of saffron carotenoids: the effect of crocin extracts and dimethylcrocetin on cancer cell lines. *Antioxidants (Basel)* 2022;11.
74. Bakshi HA, Quinn GA, Nasef MM, et al. Crocin inhibits angiogenesis and metastasis in colon cancer via TNF-alpha/NF-kB/VEGF pathways. *Cells* 2022;11.
75. Xu Q, Yu J, Jia G, et al. Crocin attenuates NF-kappaB-mediated inflammation and proliferation in breast cancer cells by down-regulating PRKCQ. *Cytokine* 2022;154:155888.

Comment 4: *Specific points.*

1. The Introduction is verbose, excessively long and not always correct. The first part (up to line 75) is very trivial, with unnecessary (and sometimes wrong) comments. This part must be

drastically reduced. Today, most new medicine are NOT from natural origin. The authors should simply focus on Saffron/crocus.

2. The objectives of the work/review are not clearly stated in the Introduction. Specify the goals and precise objective at the end of the Introduction.

Reply 4: The topic of this review is the research progress of saffron and crocin on cancer management. In the first paragraph, we briefly introduced clinical cancer management background and the limitations of currently used cancer chemotherapy. The second paragraph briefly introduced the history and importance of botanical derived compounds in cancer chemotherapy. We believe those two paragraphs are important for this manuscript, especially those in the Introduction. Nevertheless, in the revised manuscript, to more concisely introduce the background, some content in the Introduction has been deleted. To address comment #2, the objective has now been added to the Introduction.

Changes in the text: In the revised manuscript, the sentence, “To date, more the 65% of clinically used medications are originally derived from natural products (5), including but not limited to aspirin, atropine, digoxin, irinotecan, metformin, morphine, paclitaxel, and penicillin (6).” has been changed to “To date, more than 65% of clinically used medications are originally derived from natural products (5,6).”

In Introduction, the following has been added: “The objectives of this review are to present research progress in anticancer effects of saffron and crocin and the underlying mechanisms of action.”

***Comment 5:** The literature survey is largely incomplete. There are too many old references (unnecessary) and a major lack of recent references (2020-22). In particular there are recent reviews on crocin, not cited.*

Reply 5: In the revised manuscript, newly published articles have been added.

Changes in the text: The following information has been added in the revised manuscript: “Recently, saffron and crocins were found to have antiproliferative effects on glioblastoma and rhabdomyosarcoma cells (34).” and, “Recent studies demonstrated that crocin inhibits angiogenesis and colorectal cancer cell metastasis by targeting NF-κB and blocking TNF-α/NF-κB/VEGF pathways (74). Moreover, crocin inhibited NF-κB-mediated inflammation and

proliferation in cancer cells through down-regulating protein levels of protein kinase C theta (75).”

References:

34. Hatzigiapiou K, Nikola O, Marka S, et al. An in vitro study of saffron carotenoids: the effect of crocin extracts and dimethylcrocetin on cancer cell lines. *Antioxidants (Basel)* 2022;11.
74. Bakshi HA, Quinn GA, Nasef MM, et al. Crocin inhibits angiogenesis and metastasis in colon cancer via TNF-alpha/NF-kB/VEGF pathways. *Cells* 2022;11.
75. Xu Q, Yu J, Jia G, et al. Crocin attenuates NF-kappaB-mediated inflammation and proliferation in breast cancer cells by down-regulating PRKCQ. *Cytokine* 2022;154:155888.

Comment 6: *There are other important products in saffron, such as dimethylcrocetin, picrocrocetin, ... not cited.*

Reply 6: Thank you for your comments. In the revised manuscript, other important compounds from saffron, including dimethylcrocetin, have been included.

Changes in the text: The following information has been included in the revised manuscript: “Researchers also reported the inhibitory effects of dimethylcrocetin, crocetin, and crocin on human K562 chronic myelogenous leukemia cells and HL-60 promyelocytic leukemia cells with low ID₅₀, which are less than 2 μM (35,36).”

“Using different tumor cells and surgical specimens (ovarian carcinoma, fibrosarcoma and osteosarcoma), the anticancer activities of both crocin and a derivative of crocin (dimethylcrocetin) have also been observed (37).”

“Crocetin, an active compound purified from saffron, did not influence colony formation, but this compound showed significant cell growth inhibitory effects on three human cancer cell lines, including *HeLa* (cervical cancer), A-549, and VA-13 (lung cancer), via regulation of intracellular DNA/RNA and protein synthesis (33). Recently, saffron and crocins were found to have antiproliferative effects on glioblastoma and rhabdomyosarcoma cells (34).”

Comment 7: Others

Lines 67-68: More than several.... (not correct)

Lines 111-113: what mean “crocins” with an “s” (30%). Specify the other types of compounds to bring the total to 100%.

Reply 7: In the revised manuscript, those issues have been addressed.

Changes in the text: “More than several anticancer candidate compounds, such as acronyciline, bruceantin, flavopiridol, and thalicarpin, are currently under clinical evaluation (6,7).” has been changed to “Several anticancer candidate compounds, such as acronyciline, bruceantin, flavopiridol, and thalicarpin, are currently under clinical evaluation (6,7).”

“In the market, the commonly available saffron with quality control contains crocins (about 30%), picrocrocin (5 to 15%), and volatile compounds (up to 2.5%, including safranal).” has been changed to “In the market, the commonly available saffron with quality control contains crocin (about 30%), picrocrocin (5 to 15%), and volatile compounds (up to 2.5%, including safranal).”