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OPEN PEER REVIEW

The Fractions of *Eryngium thyrsoideum* Extract Sensitize Breast Cancer Cells to Apoptosis

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About this report

This open peer review (OPR) report shows the public comments made on this article during peer review, by the reviewers, the associate editor and the editor-in-chief, for every revision round, together with the authors' replies. Comments appear only with the consent of the people who wrote them. Reviewers who chose to sign their report are named; the others are shown as "Referee". Comments are edited for language only, and confidential remarks to the editors are not included.

More about open peer review: brieflands.com/knowledgebase/opr

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1 Original submission

2 comments · 5 Sep 2021 – 12 Sep 2021

Referee 1 Reviewer

MAJOR REVISION 5 Sep 2021

Dear author(s),
The submitted manuscript (#118888) needs major revisions before being considered for publication. Please consider the following comments in this regard:
1. The English language of the manuscript is very poor. There are lots of grammatical errors throughout the manuscript, so it should be rewritten in good scientific English; then it might be considered for a second review.
2. Please avoid additional explanations in the text, such as explanations of the common risk factors for breast cancer or details about the principles of the flow-cytometry technique.
3. There are multiple methodological and writing mistakes in the method section. Examples include the DMSO concentration in the stock and working solutions, the units, or the cell density at seeding, etc.
4. Please provide more details about the names of the kits or materials used, the incubation time, or the temperatures.
5. How can you describe MCF-7 cells as chemo-resistant?
6. The discussion must be revised wholly.

Editor-in-Chief

MAJOR REVISION 12 Sep 2021

Dear Authors,

Your manuscript was reviewed by three independent referees. They suggest major changes. Please revise the manuscript and submit the new version with a point-by-point response to the reviewers' comments. We will reconsider it later.

Sincerely yours,
JJNPP EIC

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Revision 1

AUTHORS' RESPONSE TO REVIEWERS

Authors' reply to reviewers

The next pages contain the authors' point-by-point reply to the comments of this revision round, as submitted with the revised manuscript. It is published as the authors wrote it.

Prof. Zahra Ramezani

Editor-in-Chief

Jundishapur Journal of Natural Pharmaceutical Products

We would like to express our appreciation to You and the esteemed Reviewers for their insightful comments, which helped us significantly to improve our manuscript. According to the suggestions, we have thoroughly revised our manuscript and its final version is enclosed. Please kindly find point-by-point responses to the comments as listed below.

Reviewers' Comments:

Reviewer 1:

The manuscript entitled “The Fractions of *Eryngium thyrsoideum* Extract Sensitize Breast Cancer Cells to Apoptosis”, Overall, this is a clear, concise, and well-written manuscript but suffers from some defects that should be addressed as follow:

Response: We thank the reviewer for this positive comment.

1-Please mention the normal cell line name correctly. HFFF (without final elements) are not available in Pasteur cell bank.

Response: With apologize, the name of human foreskin fibroblasts (HFF-2) that was purchased from the National Bank of Iran (Pasteur Institute of Iran, Tehran, Iran) was corrected in the main text.

2-in part 3.6 the unit of concentration is not correctly noted. (Micrograms are not a unit of concentration)

Response: We apologize for our mistake. All of the units of concentration were correct in the main text.

3- Are the number of cells accurately mentioned in the mL unit? How many cells are seeded in each well?

Response: In this study, 5×10^3 cells/ml were prepared and inoculated in 96-well microplates for 24 hours. As recommended by the esteemed reviewers, the sentence was revised in the “Material and method” section.

4- What is the concentration of MTT solution used in MTT assay?

Response: 20 µl of MTT solution was added to each well to reach a final concentration of 0.45 mg/ml. As advised, the sentence was edited.

5- Please mention the specifications of the ELISA reader used in MTT assay.

Response: As recommended, the specifications of the ELISA reader were mentioned.

6- It is mentioned in section 3.8 that the supernatant was then drained. Does removing the supernatant mean throwing it away? Following this statement did not mention what was isolated by trypsin. Please rewrite this sentence.

Response: The supernatant was drained and poured into a separate falcon and cells were isolated from the bottom of the well after washing with PBS by 500µl EDTA-Trypsin. As recommended the esteemed reviewers the paragraph was added in section 3.8

7- Please mention the specifications of the flow cytometer.

Response: As recommended, the specifications of the flow cytometry was mentioned.

8- In the last sentence of Section 3.8, specify which wavelength is related to the laser light used to excite the sample and which wavelength is related to the detector used to read the sample.

Response: 488 nm is excitation wavelength and 617 nm is emission wavelength. As advised, the wavelengths were added in the last sentence of section 3.8.

9- Section 3.7 states that the samples were read by ELISA Reader at 540 nm wavelengths, while Section 3.9 states that this wavelength is 570 nm. Which one is correct?

Response: With apologize for our typo mistake, the wavelength was edited

10- In extraction with organic solvents after drying the extract, very small amount of solvent remains in the sample which may have a toxic effect on the cells. Is this considered in the work?

Response: The extracts were completely dried by a rotary evaporator at 45 °C under low air pressure and after making sure the extract is completely dry, it was dissolved in DMSO solvent. The amount of DMSO was less than 0.1%, which did not have significant cytotoxic effect. DMSO control was also included in the tests to ensure this and cell viability was determined according to DMSO control. As recommended, the detail of extraction was added in “Preparation of medicinal plant powder and extraction: section.

11- Please put the figures related to the test results in the results section.

Response: As recommended, the figures were checked carefully and put the related test results in the results section.

12- add the data for the MTT test of normal cell line in the table like the other two cell lines.

Response: As advised, the data for the MTT test of normal cell (HFF-2) was added in the main text.

13-as per natural compound you are using before going for MTT assay you should check that the natural compound is not reducing the MTT and the test does not give a false positive results.

Response: According to the interpretation of flow cytometric test results of this study, the mentioned extract and fraction inhibit cancer cell growth by inducing **apoptosis**. Apoptosis is the process of programmed cell death therefore, it can be concluded the natural compound is not reducing the MTT and the test does not have the false positive results. In addition, The MTT test was carried out with negative and positive controls for detection of false results.

Reviewer 2:

Dear author(s),

The submitted manuscript (#118888) needs major revisions before being considered for publication. Please consider the following Comments in this regard:

1. The English language of the manuscript is very poor. There are lots of grammatical errors throughout the manuscript, so it should be rewritten in good scientific English, then it might be considered for a second review.

Response: We thank the respectful reviewer for useful comment. As recommended, all authors checked the manuscript in terms of unseen typo any grammatical mistakes. In addition, the manuscript has been edited by Reza Kalantari, PhD (TESOL), International English language educator and speaker. All grammar errors have been removed.

2. Please avoid additional explanations in the text. For example, explaining the common risk factors of breast cancer or details about the principles of flow-cytometry technique.

Response: As recommended the esteemed reviewers, the additional explanations of the common risk factors of breast cancer and details about the principles of flow-cytometry technique were removed.

3. There are multiple methodological and writing mistakes in the method section. For example, the DMSO concentration in the stock and working solutions, the units, or the cell density in seeding, ...

Response: We apologize for our mistake. All of the unit of concentration were correct in the main text.

4. Please provide more details about the name of the used kits or materials, the incubation time or temperatures.

Response: Thanks for your kindly suggestion, more details about the name of the used kits or materials and details about incubation time and temperatures were added in the main text.

5. How do you mention MCF-7 cells as chemo-resistant?

Response: We apologize for this mistake. The mention of MCF-7 cell line was revised.

6. The discussion must be revised wholly.

Response: We thank the respectful reviewer for the useful comment. As recommended, all authors revised the discussion section.

Reviewer 3:

Dear authors, it is a well-designed manuscript and I have some suggestions:

Response: We thank the reviewer for this positive comment.

Please explain the abbreviations.

Response: Thanks for your kindly suggestion, the abbreviations were explained at the end of the main text.

Please write the difference between two studied cancer cell line.

Response: As advised, the differences between the two cell lines MDA-MB-231 and MCF-7 were described in “Material and method” section.

line 80 page 4: Please correct “Dr. Mojallali”

Response: It was edited.

line 95 page 5: please correct the unit of concentrations.

Response: the unit of concentrations was checked carefully and edited.

Line 136 page 6: please correct the sentence.

Response: It was edited.

Line 234 page 11: breast cancer cell line.

Response: It was edited.

We hope you will find the revised manuscript suitable for publication in **Jundishapur Journal of Natural Pharmaceutical Products** and look forward to hearing from you in due course.

With Kind Regards
Yours Sincerely
Parina Asgharian, PharmD, Ph.D

2 Revision 1

1 comment · 19 Jan 2022

Editor-in-Chief

MINOR REVISION 19 Jan 2022

Dear Authors,

Our experts have scrutinized your revised manuscript and believe minor changes are required. Please revise your manuscript accordingly, and send it for further processing. We will reconsider it.
Remember, it is the last revision opportunity; therefore, your revised manuscript should satisfy the reviewers.

Yours,
JJNPP EIC

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Revision 2

AUTHORS' RESPONSE TO REVIEWERS

Authors' reply to reviewers

The next pages contain the authors' point-by-point reply to the comments of this revision round, as submitted with the revised manuscript. It is published as the authors wrote it.

Editorial Office

Jundishapur Journal of Natural Pharmaceutical Products

Dear Prof. Zahra Ramezani

We would like to express our appreciation to You and the esteemed reviewer for their insightful comments, which helped us significantly to improve our manuscript. According to the suggestions, we have thoroughly revised our manuscript and its final version is enclosed. Please kindly find point-by-point responses to the comments are listed below.

Re-Review Comments:

Reviewer 1:

Dear authors, it is a well-designed manuscript and I have some suggestions:

Response: We thank the reviewer for this positive comment.

Please mentioned the fullname of abbreviations in the text

Response: As suggested, we provided the full name of abbreviation in the text.

Please explain the abbreviations

Response: As advised, all abbreviations were explained by their full names in “Abbreviations” section.

please write the difference of these two cell line

Response: Among breast cancer cell lines, MDA-MB-231 is more sensitive than MCF-7 cell line. MDA-MB-231 cell line generally accounts for 15-20% of all breast cancer cases and is considered as a basal type of breast cancer cell line with triple-negative for estrogen (ER), progesterone (PR) and human epidermal growth factor receptor 2 (HER2) while MCF7 cell line is easily treated and is known as luminal type of breast cancer cell line with ER and PR positive. In addition, MDA-MB-231 cell line has an elongated morphology and grows rapidly but MCF-7 grows in clusters and is slow-growing. As recommended, the difference between these two cell lines was explained in “Cell Lines and Cell Cultures” section in the main text.

line 80 page 4: Please correct “Dr. Mojallali”

Response: With apologize for our typo mistake, as suggested, the correct form “Dr. Mojallali Industrial Chemical Complex Co. (Tehran, Iran).” Was added in main text, lines 80-81, page 4.

line 95 page 5: please correct the unit of concentrations.

Response: With apologize, the volume and unit of concentrations were changed to correct form and highlighted in line 102 page 5.

Line 136 page 6: please correct the sentence.

Response: The aerial parts of *E. thyrsoideum* (100 g) were extracted by soxhlet apparatus using n-Hex, DCM and MeOH solvents and reported as 3.42 g, 2.14 g and 6.15 g, respectively which are illustrated in Table 1. In addition, the results of MeOH extract’s fractions as the strongest extract are shown in Table 1. As recommended, the sentence was edited in lines 140-144 page 7.

Line 234 page 11: breast cancer cell line

Response: As recommended, the phrase was edited carefully.

Associate Editor's Comments:

The authors have made the required changes to render the paper acceptable for publication.
but still some minor revision is required.

Response: We thank the esteemed editor for this positive comment.

in Table 2-4, the significant number should be considered. for example: 146.1 ± 5.39 should be corrected as 146.10 ± 5.39. All the data should have the same number after decimal point.
or 184.00 ±5.60.

Response: With regard, the typo mistakes were edited carefully and highlighted.

We hope you will find the re-revised manuscript suitable for publication in **Jundishapur Journal of Natural Pharmaceutical Products** and look forward to hearing from you in due course.

With Kind Regards
Yours Sincerely

3 Revision 2

1 comment · 19 Feb 2022

Editor-in-Chief

ACCEPT 19 Feb 2022

Dear Authors,

It is my honor to inform you that your manuscript has been accepted for publication in JJNPP. I am sending it to the publisher for further processing.

Yours Sincerely,
JJNPP EIC