Editorial board

*Peer J*

I am enclosing the reviewed version of manuscript entitled “**Antitumor activity of *Chlorella*** ***sorokiniana* and *Scenedesmus* sp. microalgae native of Nuevo León State, México**”, by Raúl Reyna-Martinez, Ricardo Gomez-Flores, Ulrico López-Chuken, Ramiro Quitanilla-Licea, Diana Caballero-Hernandez, Julio C. Beltrán-Rocha, Cristina Rodríguez-Padilla, and Patricia Tamez-Guerra, for publication in the *Peer J* journal as original research paper. This manuscript reports the toxicity of Nuevo Leon State (Mexico) native *Chlorella* *sorokiniana* and *Scenedesmus* sp. crude organic extracts (mainly methanolic) against murine L5178Y-R lymphoma. Microalga extracts induced tumor cell death by several metabolic paths including the mechanism of apoptosis.

I would like to thank the reviewers for their careful and detailed critique of our manuscript entitled "Antitumor activity of *Chlorella sorokiniana* and *Scenedesmus* sp. microalgae native of Nuevo León State, México”. I have either replied to each specific comment addressed by the reviewer (s), or have incorporated his (her) suggestions in the revised manuscript (marked in red). I hope that this revision will answer the thoughtful and significant concerns of the reviewers.

All authors have read and approved the final submitted manuscript and affirm that contains original information, and it is not under consideration by another journal, and that no portion of this manuscript, including the abstract, has been published or posted on the internet. This manuscript contains supplementary material.

Sincerely

“*ALERE FLAMMAM VERITATIS*”

**Patricia Tamez-Guerra**

**Corresponding author**

**I would like to thank the reviewers for their careful and detailed critique of our manuscript entitled "Antitumor activity of *Chlorella sorokiniana* and *Scenedesmus* sp. microalgae native of Nuevo León State, México”. I have either replied to each specific comment addressed by the reviewer (s), or have incorporated his (her) suggestions in the revised manuscript (marked in red). I hope that this revision will answer the thoughtful and significant concerns of the reviewers.**

**In response to Reviewer 1:**

*Not enough data to support the conclusion from the study. Hence, the results are inconclusive.  
The experimental designs should be expanded to provide supporting results to justify the research question.*

*Basic reporting*

*The introduction is too brief.*

ANSWER: The introduction section was edited, as suggested (introduction section Lines 47-61 and 67-78 of the revised manuscript).

*The results for DNA degradation in 2% agarose gel electrophoresis are not found in the text or Figure.*

ANSWER: The results for DNA degradation in 2% agarose gel electrophoresis have been incorporated in the Results (Lines 282-284, Fig. 3A) and Discussion (Lines 323 to 326 and 340) sections of the revised manuscript.

*Experimental design*

*In the abstract the authors reported that the extracts were shown to induce tumor cell death by the mechanism of apoptosis. However, only one experiment was conducted (AOPI staining) to prove apoptosis (a cell death pathway). There were no experiments done to show the mechanism of apoptosis such as caspase activity and gene expression study.*

ANSWER: In addition to AOPI staining, the apoptosis mechanism was demonstrated by DNA fragmentation (Fig. 3A) and caspase activity (Fig. 3B). Caspase activity results have been incorporated in the Materials and Methods (Lines 223 to 233), Results (Lines 282 to 286), and Discussion (Lines 319 to 333) sections, as well as in Figure 3 (Lines 484 to 494) of the revised manuscript.

*Validity of the findings*

*Not enough data to prove the cytotoxic activity of the C. sorokiniana and Scenedesmus sp. methanol extracts. The results for DNA fragmentation is missing. Apoptosis should be supported by other assays such as cell cycle, Annexin 5, Mitochondrial membrane potential, caspase activity and apoptotic gene expression using qpcr.*

ANSWER: Results for DNA degradation in 2% agarose gel electrophoresis have been incorporated in the Results (Lines 282-284, Fig. 3A) and Discussion (Lines 323 to 326 and 340) sections of the revised manuscript. In addition to AOPI staining, the apoptosis mechanism was demonstrated by DNA fragmentation (Fig. 3A) and caspase activity (Fig. 3B). Caspase activity results have been incorporated in the Materials and Methods (Lines 223 to 233), Results (Lines 282 to 286), and Discussion (Lines 319 to 333) sections, as well as in Figure 3 (Lines 484 to 494) of the revised manuscript.

*The IC50 values of the extracts were not reported.*

ANSWER: The IC50 values of the *C. sorokiniana* and *Scenedesmus* sp methanolic extracts, as well as the vincristine were added to the Results section, as suggested (Lines 278 to 281 of the revised manuscript).

**In response to Reviewer 2:**

*The authors obtained water samples from a local river in Nuevo Leon Mexico and isolated two strains of micoalgae, Chlorella sorokiniana and Scenedesmus. They present details on the methods of growing and confirming the identity of these organisms by 18S PCR. Methanol extracts (250-500 ug/ml) of dried mass obtained from bioreactors was used for testing anti-cancer efficacy in mouse lymphoma L5178Y-R cells. An acridine orange/ethidium bromide (AO/EB) staining assay was used to determine effects on apoptosis and necrosis. These extracts had less cytotoxic effects on non-cancer mouse thymocyes.   
  
In general, this paper provides some interesting information on utilizing a local micoalgae organism for testing anti-cancer activity. However, there are several issues that need to be addressed.*

*1. Should provide a representative image of cells stained with AO/EB and indicate apoptotic and necrotic examples in Fig. 3.*

ANSWER: A representative image of AO/EB results was included, as suggested (Fig. 3D of the revised manuscript).

*2. The methods mention DNA laddering/smear but this figure is not presented. This is very important to provide further confidence that the extracts are working through apoptosis (laddering) more than necrosis (smear), as suggested by AO/EB results in Fig. 3. In methods line 163, should state DNA smear not scan.*

ANSWER: Results for DNA degradation in 2% agarose gel electrophoresis have been incorporated in the Results (Lines 282-284, Fig. 3A) and Discussion (Lines 323 to 326 and 340) sections of the revised manuscript. The word “scan” was changed to “smear” in the M&M section, as suggested (Line 221 of the revised manuscript).

*3. In the discussion, need to cite and comment on recent paper showing that C. sorokiniana extract inhibits lung cancer in vitro and in vivo (BMC Complement Altern Med. 2017 Feb 1;17(1):88).*

ANSWER: Lin et al (2017) paper was appropriately discussed and the reference was included in the References section (Discussion section, lines 354 to 358 of the revised manuscript).

*4. Sentence in lines 247-249 needs to be revised. It is assumed what is meant is that extracts have low cytotoxicity in non-cancer cells but higher in cancer.*

ANSWER: The sentence was edited to make the text clear (Lines 359 to 363 of the revised manuscript).

*5. English writing needs improvements.*

ANSWER: The manuscript was reviewed and English was edited for improvement, as suggested.

*6. Fig. 1, why after 18d Scenedesmus biomass much greater vs C. sorokiniana? Does not correspond to numbers given.*

ANSWER: We are in agreement with this Reviewer comment. Figure 1 results represent the biomass production data collected every 2 d during the fermentation time-course. By doing this, it seems that *Scenedesmus* sp. biomass production in the photobioreactor will result in twice as much as compared with that produced by *C. sorokiniana*, since the highest dried value from samples were higher than 0.8 and up to 0.4, respectively (Fig. 1). However, after collecting all the final biomass of each microalgae, *Scenedesmus* sp. produced 18% more compared with *C. sorokiniana*, for a total of 13.74 g and 11.21 g dried biomass, respectively. This statement was included in the Discussion section of the revised manuscript (Lines 301 to 308).

*7. Several issues in methods section: a) more information of 18S PCR to identify strains; b) “Tumor cytotoxicity and apoptosis assay” subheading is misplaced and should be moved to line 120; c) how is the concentration of the extract measured? It is given as 1 mg/ml; d) more information on microscope source, wavelength used for analysis; e) there is mention of vincristine as positive anti-cancer drug but actinomycin D is used in Fig. 3.*

ANSWER:

1. More information of 18S PCR to identify *C. sorokiniana* was included in the Materials and Methods section of the revised manuscript (Lines 106 to 119).
2. Tumor cytotoxicity and apoptosis assay subheading was moved, as suggested (Line 180 of the revised manuscript).
3. The information of the microalgae extract concentration measurement was included in the Materials and Methods section of the revised manuscript, and indeed the stock solution was prepared at 1 mg/ml (Lines 168 to 173 of the revised manuscript).
4. Information of microscope source and wavelength used for the analysis was included in the Materials and Methods section of the revised manuscript (Lines 204-205, and 230 to 233).
5. Vincristine was used as positive anti-cancer drug (Lines 186 to 187 of the revised manuscript), whereas actinomycin D was used as positive control for apoptosis (Lines 214, and 228 to 229 of the revised manuscript).

*Basic reporting*

1. *English writing needs improvements.*

ANSWER: The manuscript was reviewed and English was edited for improvement, as suggested.

1. *In the discussion, need to cite and comment on recent paper showing that C. sorokiniana extract inhibits lung cancer in vitro and in vivo (BMC Complement Altern Med. 2017 Feb 1;17(1):88).*

ANSWER: Lin et al (2017) paper was appropriately discussed and the reference was included in the References section (Discussion section, lines 354 to 358 of the revised manuscript).

1. *Should provide a representative image of cells stained with AO/EB and indicate apoptotic and necrotic examples in Fig. 3.*

ANSWER: A representative image of AO/EB results was included, as suggested (Fig. 3D of the revised manuscript).

1. *The methods mention DNA laddering/smear but this figure is not presented. This is very important to provide further confidence that the extracts are working through apoptosis (laddering) more than necrosis (smear), as suggested by AO/EB results in Fig. 3. In methods line 163, should state DNA smear not scan.*

ANSWER: Results for DNA degradation in 2% agarose gel electrophoresis have been incorporated in the Results (Lines 282-284, Fig. 3A) and Discussion (Lines 323 to 326 and 340) sections of the revised manuscript. In addition to AOPI staining, the apoptosis mechanism was demonstrated by DNA fragmentation (Fig. 3A) and caspase activity (Fig. 3B). Caspase activity results have been incorporated in the Materials and Methods (Lines 223 to 233), Results (Lines 282 to 286), and Discussion (Lines 319 to 333) sections, as well as in Figure 3 (Lines 484 to 494) of the revised manuscript.

The word “scan” was changed to “smear” in the Materials and Methods section (Line 220 of the revised manuscript).

*Experimental design*

1. *Appears to be original in that they obtained organisms from local river, However, there is one very recent but more complete paper mentioned above that uses C. sorokiniana extract in lung cancer.*

ANSWER: We were not aware of Lin et al. (2017)´s paper. Nevertheless, our findings support this report.

1. *Methods for isolation, growth, and preparation of extracts is well described. There are some issues in methods described in general comments.*

Answer. All general comments were addressed as suggested.

*Validity of the findings*

1. AO/EB results would benefit from a representative image.

ANSWER: A representative image of AO/EB results was included (Figure 3D of the revised manuscript).

1. *DNA laddering result is not shown and is essential before acceptance.*

ANSWER: Results for DNA degradation in 2% agarose gel electrophoresis have been incorporated in the Results (Lines 282-284, Fig. 3A) and Discussion (Lines 323 to 326 and 340) sections of the revised manuscript.