Author’s response to reviews

Title: In Vitro Anti-proliferative Activity of Argemone gracilenta and Identification of Some Active Components

Authors:

Juan C Galvez-Ruiz (anisolborano@guayacan.uson.mx)
Mario A Leyva-Peralta (mario_ups@hotmail.com)
Ramon E Robles-Zepeda (rrobles@guayacan.uson.mx)
Adriana Garibay Escobar (agaribay@guayacan.uson.mx)
Laura P Alvarez-Berber (lalvarez@uaem.mx)
Eduardo Ruiz-Bustos (erbustos@guayacan.uson.mx)

Version: 3 Date: 4 September 2014

Author’s response to reviews:

Reply to reviewer

We thank the reviewer for the comment and apologize for the several errors we have done. Now we have reworded some sections in order make the paper easier to read and many of reviewer’s doubts have been answered. The 1H and 13C data are now well assigned. And the manuscript has been revised by a native English speaker.

Reviewer: Jiang Hu
Comment:
.., the literatures related to the chemical composition should be cited, such as ‘Schwarzbach et al., Plant Systematics and Evolution, 1999, 218(3-4), 257-279; Stermitz et al., Journal of Organic Chemistry (1969), 34(3), 555-9,'...
Answer
Done
Comment
..furthermore, both the structure and the biological activity of the compounds were not interesting enough. Overall, this manuscript seems not bring enough knowledge or innovation,....
Answer
The point of view of the reviewer is not completely share by the other two reviewers. The results are very interesting because it is the first time that the anti-proliferative properties of argemonine has been described and these properties are similar to some drugs currently used. This can be the source of some more active compounds prepared from this alkaloid.

Reviewer: Rémy Bertrand TEPONNO
Comment
There are serious problems on the 1H and 13C data of Argemonine. There is certain symmetry in the molecule but I don’t understand why the 1H and 13C chemical shifts of positions C-5 (33.3 ppm) and C-11 (56.3 ppm) are quite different. Furthermore, C-8 and C-9 are two aromatic carbons bearing oxygen: they are supposed to appear around 145 ppm such as C-2 and C-3. The authors should have to look again on the 1H and 13C spectra of their compound and compare them to the spectroscopic data of Argemonine present in the literature.

Answer
We apologize for the several mistakes we have done. We have revised the 1H and 13C spectra and the data are now correct.

Comment:
2- The 13C chemical shift of Argemonine should be given with one decimal part.
Answer:
Done

Comment:
3- The Rf is used to characterize a pure compound. Since fractions FAg-4A and FAg-5B are mixtures of at least 4 compounds each, I don’t think that it is good to characterize them by their Rf. Even if it is not possible to separate them with column chromatography, the thin layer chromatography can show that they are mixtures of compounds of very similar polarities.

Answer:
Agree. We have reworded some sentences.

Comment:
4- The authors should explain why they said that the EtOAc fraction was the most active (line 106). In table 1, it is evident that the Hexane fraction was the most active when comparing the IC50 values.

Answer:
We consider that the EtOAc fraction was the most effective one, because it is very active against the cancerous cell lines and almost no active against the normal cell line. The hexane fraction is active against both cancerous cell lines and normal cell line. So the main goal is to get a fraction or compound that could display a high anti-proliferative activity against cancerous cell lines and no activity against normal cell lines.

Comment
5- On line 120, instead of "13C NMR (CDCl3, 400 MHz)", the authors should write "13C NMR (CDCl3, 100 MHz)". On line 128, instead of "13C NMR (CDCl3, 400 MHz)", the authors should write "13C NMR (CDCl3, 100 MHz)

Answer
Done
Reviewer: Horacio Olivo

Comment:

Figure 2 shows the structure of argemonine and presents the 1H and 13C NMR signals. They are not well assigned. The molecule has a plane of symmetry. The signals at C8 and C9 are wrong. A 1H NMR of argemonine has been reported in the literature (Johnson, A. P. J. Chem. Soc. Perkin Trans 1 1996, 907). The signals in the literature do not match the chemical shifts reported by the authors. The N-Me signal appears at 2.54 ppm (page 5, line 117), but in the Figure is reported as 2.45. The authors mention in the text that 1H and 13C NMR spectra was identical to the one reported in the literature (Marazano, JOC 2004). Actually, in Marazano’s paper, they make the correct assignment of the NMR signals of argemonine! This figure needs to be corrected.

Answer

We apologize for the several mistakes we have done. We have revised the 1H and 13C spectra and the data are now correct.

Comment:

IC50’s of 2.0 to 10.0 should not be considered “high” anti-proliferative activity. This is moderate activity. The IC50 value of 79.5 mg/mL should be considered inactive.

Answer:

The United States National Cancer Institute (US-NCI) establishes that a crude extract that shows an IC50 value of less than 100 µg/mL is considered active. When the IC50 value is lower than 30 µg/mL, the US-NCI considers a crude extract promising for purification and a biological activity study [3,23]. In the case of pure compounds, they are considered active when the IC50 values are lower than 4 µg/mL [21, 22].

Comments:

Page 3. Line 57. The words “cardo or chicalote” need to be in between quotation marks (these are words in other language).

Page 3. Line 58. Replace “showed” by “shown”

Page 4. Line 92. Remove “Eng” before the name Jesus.

Page 4. Last line. What do the authors mean by methanol (1:10 w/v)? Was this a mixture of solvents? Methanol-water? Water-methanol?

Page 5. Replace “(3 X 400 mL)” for “(3 X 400 mL for each solvent)”

Page 5. Lines 106-108. What was the ratio of the hexanes-DCM-methanol mixture?

Page 5. Line 118. dd needs to have two coupling constants.

Page 5. Line 100. Remove “(Ruiz et al., 2009)” This is not the proper note for references.

Pages 5 and 6. The carbon chemical shifts are usually reported with just one decimal digit, not two.
…“Phytochemicals have always been studied because of their inherent potential to cure diseases, as demonstrated by ancient medicinal practices.” I do not agree with this sentence. Not all phytochemicals have an inherent potential to cure diseases and they have not always been studied because of that reason. Many phytochemicals have no activity in humans, and some of them might be too toxic to be used by humans (strychnine for example).

Answer:
Agree, we have reworded the paragraph.

Comment:
The MTT test is an old method for determination of cell viability. More reproducible essays are now available (see CCK-8 from Sigma-Aldrich).

Answer:
We really appreciate the comment from the reviewer and we have learned an easiest way to perform the experiments. It is not possible for us to afford the reagents to carry out all of the experiments again, but we are going to use the CCK-8 method in future works.

Comment:
The criterion to establish that fractions are “very active” if < 10 ug/mL, “active” for >10 and <50 ug/mL, “moderately active” if >50 and 100 ug/mL, and >100 ug/mL are non-active is very arbitrary. Currently, <10 ug/mL is considered moderately active, and nM is considered very active. The reference the authors cite (reference 21) is not from an authoritative research group and is not from a high impact journal. The authors should be more critical when reading articles from low-impact journals.

Answer:
We appreciate the comment and we have used the data available in the US-National Cancer Institute.