Author's response to reviews

Title: Phytochemical Constituents and Medicinal Properties of Different Extracts of Strobilanthes crispus (L.) Bremek Grown in Different Locations of Malaysia

Authors:

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Author's response to reviews: see over
Dear Editor BMC Complementary and alternative medicine

MS: 1965416161746738

Phytochemical Constituents and Medicinal Properties of Different Extracts of *Strobilanthes crispus* (L.) Bremek Grown in Different Locations of Malaysia

We did all corrections based on reviewer coments. List of corrections are as bellow:

**Reviewer 1, Jaures K Noumedem**

<table>
<thead>
<tr>
<th>NO</th>
<th>COMMETS</th>
<th>CORRECTIIONS</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>The whole manuscript has to be edited as there are numerous typos</td>
<td>Corrected and manuscript was sent for English editing. Certificate of English editing is attached.</td>
</tr>
<tr>
<td>2</td>
<td>The authors should make a better search for the existing information. Few omissions include: Line 97-98 The authors claim that: extraction or high-performance liquid chromatography (HPLC) analysis techniques have not previously been developed for this material. They should check for the thesis entitled pharmacological evaluation of <em>Strobilanthes crispus</em> (L.) blume written by CHIN LEE CHENG since 2008. They should also consider the findings of this thesis as far as the anticancer activity of <em>Strobilanthes crispus</em> leaf extract is concerned. Consider also the study: <em>Strobilanthes crispus</em> Extract Induces Apoptosis through Enhanced Caspases Activities in Cervical Cancer Cells. Yen Hoong Chong et al. This study should be consider also in the discussion section.</td>
<td>Corrected ad we removed this sentence from the manuscript. But, our mean from this sentence was extraction or high-performance liquid chromatography (HPLC) analysis techniques have not previously been developed for <strong>flavonoids</strong> and <strong>phenolic acids</strong>. The thesis that you referee (LEE CHENG 2008) is not about flavonoids and phenolic acids. Based on your comment we used these references in anticancer section. This study was added in anticancer section as references.</td>
</tr>
<tr>
<td>3</td>
<td>Title: - give precision on the fact that extracts are from leaves</td>
<td>Corrected in the title.</td>
</tr>
<tr>
<td>4</td>
<td>Line 43 and 44: change ‘various solvents (aqueous and ethanol)’ into aqueous and ethanol extracts</td>
<td>Corrected.</td>
</tr>
<tr>
<td>5</td>
<td>Line 45: change ‘Aqueous extracts’ into Aqueous extract</td>
<td>Corrected.</td>
</tr>
<tr>
<td>6</td>
<td>Line 46: ‘TPC (12.62 mg/g DM)’ : give the signification of ‘DM’ as it is the first time it appears in the manuscript</td>
<td>Corrected.</td>
</tr>
<tr>
<td>Line</td>
<td>Original Text</td>
<td>Corrected Text</td>
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<tr>
<td>7</td>
<td>Line 50: change ‘were phenolic acids:’ into were phenolic acids classified as followed.</td>
<td>Corrected.</td>
</tr>
<tr>
<td>8</td>
<td>Keywords: why do the authors separate some keywords using comma (,)? and the others using semi colon (;)?</td>
<td>Corrected all to ;.</td>
</tr>
<tr>
<td>9</td>
<td>Delete “UHPLC” since the authors are not studying the methods</td>
<td>Method is available at page 7 line 158.</td>
</tr>
<tr>
<td>10</td>
<td>At least a general statement should be said about cancer and oxydation or antioxydants as well as the relationship between cancer and oxydation</td>
<td>Corrected and some sentences were added.</td>
</tr>
<tr>
<td>12</td>
<td>Line 170: ‘Leaf extract (100 µL) and deionized’</td>
<td>Create a space between ‘(100 µL)’ and ‘and’.</td>
</tr>
<tr>
<td>13</td>
<td>Line 171: Change ‘ ’ into 37 °C.</td>
<td>Corrected.</td>
</tr>
<tr>
<td>14</td>
<td>Line 187-188: Change ‘The concentration of extracts used ranged between 20, 40, 80, 160, 320, and 640 µg/mL’ into the following concentrations of extracts were used: 20, 40, 80, 160, 320, and 640 µg/mL.</td>
<td>Corrected.</td>
</tr>
<tr>
<td>15</td>
<td>Line 190: Each point represents the mean of triplicate experiments’ put this sentence at the end of the paragraph. Before this sentence, the authors should mention the graph that was plotted.</td>
<td>Corrected.</td>
</tr>
<tr>
<td>16</td>
<td>Line 199: ‘concentrations of TPC and the values were also dependent on the solvent’ : values of what?</td>
<td>Corrected and “value” was removed.</td>
</tr>
<tr>
<td>17</td>
<td>Line 210: is it ‘TPC’ or TFC?</td>
<td>Corrected to TFC.</td>
</tr>
<tr>
<td>18</td>
<td>Line 241: Change ‘potent free radical inhibition’ into better free radical inhibition</td>
<td>Corrected.</td>
</tr>
<tr>
<td>19</td>
<td>Line 242: Change ‘Thus, the results indicated that aqueous extracts have superior antioxidant activity’ into Thus, the results indicated that aqueous extracts have higher antioxidant activity</td>
<td>Corrected.</td>
</tr>
<tr>
<td>20</td>
<td>Line 247-249: ‘Qader et al. [7] reported that aqueous extracts of S. crispus leaves (1mg/mL) showed antioxidant activity with Fe2+ reducing ability (1182 mM/g) compared to gallic acid (1216.67 mmol/g) using the FRAP assay’. Do the</td>
<td>No, this is only example of previous work which is done for FRAP activity of S. crispus.</td>
</tr>
<tr>
<td>Line/Item</td>
<td>Suggested Changes</td>
<td>Corrected Changes</td>
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<td>-----------</td>
<td>------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------</td>
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<tr>
<td>21</td>
<td>Line 309: give the signification of IC50 as far as anticancer activity is concerned</td>
<td>Corrected.</td>
</tr>
<tr>
<td>22</td>
<td>Line 334-335: Delete ‘if the three different sampling locations from northeast (Kelantan) to north-west (Penang) are compared’</td>
<td>Corrected.</td>
</tr>
<tr>
<td>23</td>
<td>Line 337: Delete ‘One of the significant findings of this study is that’ Titles of the tables</td>
<td>Corrected.</td>
</tr>
<tr>
<td>24</td>
<td>Table 2: why the authors did not consider statistical analysis for IC50? They should also include BHT and a-tocopherol when considering statistical analysis</td>
<td>Corrected, std value of IC50 was added and BHT and a-tocopherol were considered in statistical analysis.</td>
</tr>
<tr>
<td>25</td>
<td>The authors should be more concised and more specific</td>
<td>Corrected.</td>
</tr>
<tr>
<td>26</td>
<td>Change ‘A.Rahmat was participated’ into ‘A. Rahmat participated’</td>
<td>Corrected.</td>
</tr>
<tr>
<td>27</td>
<td>Many abbreviations are used in the manuscript, it is better to include a list of abbreviations before competing interests and authors’ contributions</td>
<td>Corrected and list of abbreviation was added before competing interests.</td>
</tr>
<tr>
<td>28</td>
<td>The chemical composition study is focused on phenolic acids, saponins and flavonoids: the authors should explain why they focus the chemical composition study only on these component</td>
<td>Of cures in biochemical analysis work, we can consider a lot of compounds but there are several limitation in this case. We already test for some other compounds (some alkaloids, tannins and fatty acids) but they were not presence or presence with little concentration in <em>S.crispus</em> samples. Our working team are member of Malaysian herbal monograph committee which are working on develop of Malaysian monograph (available in GLOB in MED website: <a href="http://www.globinmed.com/index.php?option=com_content&amp;view=article&amp;id=80849&amp;Itemid=101">http://www.globinmed.com/index.php?option=com_content&amp;view=article&amp;id=80849&amp;Itemid=101</a>). According to the previous work with other authors and according to the published information in Malaysian herbal monograph site (GLOB in MED) <em>S.crispus</em> is rich in flavonoids and phenolics like catechin, epicatechin, rutin etc. We test aqueous and ethanol because of other solvents (petroleum, acetone, methanol, etc) have toxic effect. We have in vivo test following to this work and we</td>
</tr>
</tbody>
</table>
need to feed the plant extract to mice in order to test anticancer and antidiabetic activity of *S.crispus* which is necessary to do water and ethanol extraction. Some of our published monograph works are available in below address:


<table>
<thead>
<tr>
<th>NO</th>
<th>COMMETS</th>
<th>CORRECTIOS</th>
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<tbody>
<tr>
<td>1</td>
<td>One of the main objectives of this study is to evaluate the activity of the plant grown in different areas, but there is limited information available on the sampling site and condition except the state name. The sampling location should be presented in detail. Instead of only mentioning the state name, it would be better if authors can name the exact location of the area and the geographical surface/environment/habitat of the location. Authors should highlight the collection stage that has been selected for the study, e.g., the flowering stage or plant development. This will add more valid and valuable information for this study and as supportive data for discussion.</td>
<td>Climatic and geographical information of sampling locations were added. Harvesting stage was added.</td>
</tr>
<tr>
<td>2</td>
<td>Presented in line 293-295 should be presented earlier in the text and summarized in a table with other data on the sampling condition. This table can aid the reader to do a brief comparison on the three different areas where the plant grows.</td>
<td>Corrected based on comment. These data were transferred to Table 1.</td>
</tr>
<tr>
<td>3</td>
<td>Authors also suggested having one separate paragraph (line 289-305) at the end of discussion to discuss on how different habitat affect the biological activity and phytochemical content of the species and to discuss more details on this matter rather than just a brief discussion.</td>
<td>Corrected.</td>
</tr>
<tr>
<td>4</td>
<td>The MTT in vitro cell proliferation assay is one of the assays for evaluating preliminary anticancer activity and to measure the cell viability and proliferation. The term anticancer activity used in this study is too general and did not reflect the mechanism of the assay. Authors are suggested to replace the term anticancer activity with anti proliferative activity/effect. The changes are applied throughout the manuscript.</td>
<td>Corrected based on the comment.</td>
</tr>
<tr>
<td></td>
<td>Original Text</td>
<td>Corrected</td>
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<tr>
<td>5</td>
<td>Is HeLa is breast cancer or cervical cancer cell? In the method, HeLa was</td>
<td>Is cervical cancer cell, typing error was happen and the sentence was corrected.</td>
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<tr>
<td></td>
<td>referring to breast cancer cell. Please check on this and provide the source</td>
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<td></td>
<td>of the cell line. Check the HeLa spelling in entire text</td>
<td></td>
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<tr>
<td>6</td>
<td>For both DPPH and FRAP assay, authors was referring to the activity as</td>
<td>Corrected in the text and Table 3 based on reviewer comments</td>
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<tr>
<td></td>
<td>DPPH activity and FRAP activity. DPPH is a chemical name and do not reflect</td>
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<td>what type activity taking place. One of the reason to have more than one</td>
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<td>antioxidant assay is, each assay represent different mechanism. Authors</td>
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<td></td>
<td>are advised to refer to the mechanism, e.g. for DPPH assay the activity</td>
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<td></td>
<td>should refer as scavenging activity/effect and reducing activity for FRAP.</td>
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<td></td>
<td>Thorough revision is required including Table 3. Specified the activity in</td>
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<td></td>
<td>Table 3.</td>
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<tr>
<td>7</td>
<td>In discussion, authors make the comparison of <em>S. crispus</em> with other plant</td>
<td>Corrected and some of previous works on antioxidant activity of <em>S. crispus</em> was added. In manuscript we did not compared antioxidant activity of S.crispus with other plants. Just we report: Several studies reported a significant correlation between the antioxidant activity of herbs and the phytochemical content. All of our references for antioxidant activity are for same plant.</td>
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<td>species. There are quite a number of works had been reported on the</td>
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<tr>
<td></td>
<td>antioxidant activity and phytochemical profile of <em>S. crispus</em>. Authors are</td>
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<td>suggested to make the comparison within the same species and this will be</td>
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<td>more valid discussion on the factors that contribute to the differences in</td>
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<td>the data.</td>
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<tr>
<td>8</td>
<td>The references are not according to the format of the journal. Refer to the</td>
<td>Corrected. References were arranged using EndNote software based on format of journal.</td>
</tr>
<tr>
<td></td>
<td>instructions to authors. Scientific name should be in italic.</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>Authors are suggested to revised the title...medicinal properties replaced</td>
<td>Corrected I title.</td>
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<td></td>
<td>with biological activities.</td>
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<tr>
<td>10</td>
<td>Line 43: Remove the term various solvents. Only two solvents are used in</td>
<td>Corrected.</td>
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<td>this study.</td>
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<tr>
<td>11</td>
<td>Line 52-55: The term potent activity is not suitable. Extract from Kelantan</td>
<td>Corrected.</td>
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<tr>
<td></td>
<td>did show some activity, but is low when compared to the tamoxifen, Revised</td>
<td></td>
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<td></td>
<td>line 52-55.</td>
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<td></td>
<td>Description</td>
<td>Correction</td>
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</tr>
<tr>
<td>12</td>
<td>SI requires a space to be used to separate the unit symbol from the numerical value.</td>
<td>Corrected.</td>
</tr>
<tr>
<td>13</td>
<td>The abbreviation of the genus name (S.) should be in the same row with the species name (crispus</td>
<td>Corrected.</td>
</tr>
<tr>
<td>14</td>
<td>Line 213 &amp; 246: check spelling of Kelantan</td>
<td>Corrected.</td>
</tr>
<tr>
<td>15</td>
<td>Table 3: What does ND represent?</td>
<td>Corrected to ND represent not detected.</td>
</tr>
<tr>
<td>16</td>
<td>Figure 2 &amp; 3: Check the spelling of Tamoxifen</td>
<td>Corrected.</td>
</tr>
<tr>
<td>NO</td>
<td>COMMENTS</td>
<td>CORRECTIONS</td>
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</tr>
<tr>
<td>1</td>
<td>Abstract: there is statement that compounds were ISOLATED, whereas in the methodology and results is no mention about any isolation process</td>
<td>Our mea was identification. We removed “Isolation” and corrected in whole of manuscript to identification.</td>
</tr>
<tr>
<td>2</td>
<td>Methods: Preparation of extract – the final value of extract obtained after evaporation is not written, maybe it was dry residue? In this case, the amount should be stated.</td>
<td>Corrected and crude extract weight were added.</td>
</tr>
<tr>
<td></td>
<td>There is no mention about method/test used for total saponin determination Separation and identification section. On what basis the identification was done? No info. Were any standards used?</td>
<td>Corrected in methodology section.</td>
</tr>
<tr>
<td></td>
<td>DPPH section. % of inhibition was calculated for each concentration of extract?</td>
<td>Yes.</td>
</tr>
<tr>
<td></td>
<td>Anticancer activity. The cell lines were purchased from...?</td>
<td>Corrected and related information was added.</td>
</tr>
<tr>
<td></td>
<td>Any mention of suppliers of reagents used in the whole manuscript (not only Anticancer)</td>
<td>Information regarding purchase place of chemicals was added in HPLC, antioxidant and anticancer section.</td>
</tr>
<tr>
<td>3</td>
<td>Section named: Isolation and identification. Do you mean separation and identification? There is no mention of any isolation process</td>
<td>We removed “isolation” from whole of manuscript.</td>
</tr>
</tbody>
</table>
CERTIFICATE OF ENGLISH EDITING

This document certifies that the paper listed below has been edited to ensure that the language is clear and free of errors. The edit was performed by professional editors at Editage, a division of Cactus Communications. The intent of the author’s message was not altered in any way during the editing process. The quality of the edit has been guaranteed, with the assumption that our suggested changes have been accepted and have not been further altered without the knowledge of our editors.

TITLE OF THE PAPER
Phytochemical Constituents and Medicinal Properties of Different Extracts of Strobilanthes crispus (L.) Bremek grown in different locations of Malaysia

AUTHORS
ALI GHASEMZADEH

JOB CODE
MAJAA_8

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Yours sincerely,

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