Reviewer's report

Title: Antioxidant, antibacterial, cytotoxic and apoptotic activity of extracts of Cephalotaxus griffithii Hook. f. (stem bark)

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Reviewer: Nripendranath Mandal

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Title: Antioxidant, antibacterial, cytotoxic and apoptotic activity of extracts of Cephalotaxus griffithii Hook. f. (stem bark)

Comments:
This is an average study, provided with very preliminary information regarding the antioxidant, antibacterial, cytotoxic and apoptotic activity of the plant Cephalotaxus griffithii. The present study is not clarified properly to justify its therapeutic properties. So, there are certain major revisions required for this article. Most of the points to be checked and rectified are highlighted in the pdf version of the manuscript. The major concerns are summarized below.

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Major Compulsory Revisions:
1. The interest of the study, if focused on the antioxidant property, should be concentrated in much more common antioxidant and various radical scavenging assays, viz. hydroxyl radical and nitric oxide radical scavenging with the samples (See Hazra et al., 2008. BMC Complementary and Alternative Medicine 8:63).
2. The different concentration range used for the assays is to be mentioned in the manuscript. The unit of the reagents should be represented in molar concentration (M or mM).
3. The solvent in which the isolated extract has been dissolved should be clearly mentioned and if the extracts are dissolved in the same respective solvent then negative control using that particular solvent should be mentioned in antibacterial as well as in cytotoxicity assay.
4. It is mandatory to show the obtained extracts have no cytotoxic effect on normal cells.
5. Since there is an abrupt decrease of the cell viability from 40 to 80 µg/ml in the cytotoxicity assay, the concentration range should be expanded to get some intermediate values such that the IC50 value for the activity of the extract can be calculated appropriately.
6. The flow cytometry assay should be done in dose dependent manner. The data obtained is not enough to say the extracts are inducing apoptosis; it is only showing cell death.
7. In DNA fragmentation assay, the cell culture condition during extract treatment is to be mentioned. Image for Figure 6 is not appropriate, and the DNA appears to be smeared. For an ideal DNA ladder image, check Biochemical Pharmacology 81 (2011) 891-909.

8. In fluorescence microscopy, the procedure for the differential staining with EB and AO should either be mentioned and/or a reference is to be provided for the same.

9. Explain and/or provide reference for the procedure to determine ‘bacterial cell suspension containing 1×10^6cfu/ml’.

10. In the results of Antioxidant activity, explain why PE was not evaluated for the super oxide radical scavenging assay?

“PE extract treatment was not obtained because of precipitation” Explain which type of precipitation? Is the precipitation having any interference in spectrophotometric reading? It can be done by centrifugation of the reaction mixture and take the OD from the supernatant.

11. In the results section of Antibacterial activity:

# The antimicrobial activity should be done in dose dependent manner.

# The results for the assay should be mentioned thoroughly in the result section. Zone of inhibition of a particular test microbe exhibited by a specific extract must be mentioned.

# According to the table positive control did not inhibit Staphylococcus aureus. So, provide appropriate positive control which will inhibit all the test bacteria.

# Picture showing zone of inhibition can be included as a proof for antibacterial studies.

# Explain why some extracts was not tested for MIC?

# MIC result should be mentioned against particular microbes

12. How do you calculate TFC being 2.5 fold higher then quercetin, while it is use for the standard graph?

13. A large number of language, syntax and construction errors are there in the manuscript, which should be revised thoroughly. The manuscript must be copyedited before being accepted for publishing. Until and unless it is checked thoroughly, the manuscript may be liable for rejection.

Minor Essential Revisions:

1. If any ritual/traditional use of this plant part is available please include in the background.

2. In cytotoxicity assay the control should be mentioned.

3. Centrifugation speed in all procedures is to be mentioned in terms of ‘g’

4. In representing the flow cytometry data, histogram of cell cycle analysis should be provided.

5. In the result for Total Phenolic content, the range is not in accordance to the
6. “Among the tested extracts, ACE extract…… from one another” – Reconstruct the sentence.

7. “The SBCG extracts…..Staphylococcus aureus.” According to the sentence all extracts inhibited all the test bacteria. So, rewrite the sentence according to the result.

8. Tables must comprise of a heading and the legend for that table is to be given as a footnote properly and with clarity. The three tables provided as supplementary files are to be merged and formatted appropriately. It is better if the findings as described in the first two tables are correlated with the respective antioxidant activity.

9. Units for everything should be uniform throughout the manuscript.

10. The reference should be checked thoroughly for uniform and in prescribed format.

**Level of interest:** An article whose findings are important to those with closely related research interests

**Quality of written English:** Not suitable for publication unless extensively edited

**Statistical review:** No, the manuscript does not need to be seen by a statistician.

**Declaration of competing interests:**

I declare that I have no competing interests