Reviewer's report

Title: Edible bird's nest ameliorates oxidative stress-induced apoptosis in SH-SY5Y human neuroblastoma cells

Version: 2

Date: 28 July 2014

Reviewer: Audrey Yong

Comment: The authenticity of EBN is imminent. Provide evidence of authentication (Certificate of authenticity) of EBN.

Feedback: Authentication of EBN is not a standard procedure practiced in Malaysia currently, this is made evident by studies of other research teams in Malaysia (see references below), which did not provide any authentication information as well. However, we have provided information on the origin and bird species from which the raw EBN is produced. (Page 6, 1st paragraph, line 1)

References:
1. Effects of edible bird's nest (EBN) on cultured rabbit corneal keratocytes.
2. Effect of Edible Bird's Nest on Caco-2 Cell Proliferation
3. Effect Of Edible Bird's Nest On Cell Proliferation And TNF-# Release

Yong's response: Authentication of natural products is of utmost importance in natural product research. This is to assure that the test product that is research upon is indeed what it should be, in this case edible bird nest. In the 3 reference quoted, the authors researched natural product obtained from national institutes or national agency which assure the traceability of its origin. On the other hand the author of the current manuscript is not able to provide such evidence of authenticity. It looks like the researcher has taken this aspect very lightly.

Comment: The EBN was first prepared into pancreatin-digested crude extract (S1) and water extract (S1) were water soluble samples. Method of extraction does not reflect how it is traditionally prepared.

Feedback: Author has elaborated on rationale of pancreatin digestion in methodology. (Page 6, 2nd paragraph, line 2)

Yong's response: Traditional preparation of bird nest should include high temperature aqueous extraction before pancreatin digestion methodology. Birdnest is used as a nutritious food product for long time, therefore when researching this; the methodology should closely reflect how it is traditionally prepared.
Comment: Test materials, S1 and S2 are aqueous extracts, these samples should easily dissolved in deionised water (followed by filtration). This manuscript described the polar samples S1 and S2 test solutions were made in DMSO, a relatively non polar solvent. Sonication of 6 hours was required to increase solubility. Sonication of this length is not usual and may disrupt the integrity/quality of the bioactive component.

Feedback: Justification-The method to prepare EBN extracts S1 and S2 in DMSO followed by sonication was adopted based on several considerations described below:

• DMSO is a solvent able to dissolve polar and nonpolar small molecules. S1 was not a product of aqueous extraction of EBN but crude extract. Considering that it might contain both polar and non-polar bioactive compounds, and that it may not be fully solubilized in distilled water, thus we decided to use a single universal solvent system to prepare the stocks for both S1 and S2 extracts.

• In order to maximize solubility of the product, sonication was applied and stopped as soon as the product was seen completely dissolved.

Yong’s response: S1 is pancreatin-digestion extract, which is not non-polar solvent extract, therefore S1 is an aqueous extract. This should dissolve easily in water. All aqueous extract should contain minimal non-polar small molecules. Was water used as a solvent in the first place?

Comment: Figure 2 Cell micrographs (B, Bi, Bii and Bii) are of different contrasts. The arrow head used are of inconsistent shapes and size.

Feedback: Author has replaced Figure 2 with new figure. Methodology (Page 8-9, 3rd paragraph) and results (Page 12, 1st & 2nd paragraph) is elaborated on nuclei staining using DNA stain Hoechst 33258 to evaluate apoptotic features in cells.

Yong’s response: Noted.

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

**Quality of written English:** Acceptable

**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