Author’s response to reviews

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

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Author’s response to reviews: see over
Dear editor,

We attached hereby the feedback to reviewer’s comment. Please refer to below for point-by-point response to the concerns raised by Dr. Audrey Yong.

Thank you.

Regards,
Dr. Ng Khuen Yen
Corresponding author
Reviewer: Audrey Yong

**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.

**Feedback:** Unlike study using plant extract, which the researcher can routinely submit a dried plant sample to the herbarium for authentication, there is currently no authentication procedure in place for EBN (an animal source extract) in Malaysia. Hence we are unable to provide this information. EBN in this study is directly obtained from swiftlet farmer from Perak state. The origin and information of the EBN are already included in the manuscript.

**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.

**Feedback:** Our team did not intend to prepare EBN in the “traditional” way. Instead we have adopted methodology by Guo et. al. because several studies which have reported observable beneficial effects of EBN (i.e. anti-osteoporosis and anti-influenza) have been using the similar preparation. Hence we wish that our study design would also produce comparable bioactivity if we prepare EBN extract similarly. However we do agree with the reviewer’s view that this study might have limitation in extrapolating the findings to the bird’s nest dietary consumption prepared by the traditional method.

**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?

**Feedback:** In the preparation of S1, cleaned bird’s nest was dried and grounded into fine powder before being digested with pancreatin. Water was added subsequently to solubilize the pancreatin powder rather than to serve the purpose of dissolving the EBN powder because raw EBN is not water-soluble in nature. We use raw EBN powder (instead of aqueous extract only) for cell treatment because we wish to retain as much bioactivity of EBN as possible. However we did include the aqueous extract in the study as S2. This aims to investigate if the bioactivity is contributed by the water fraction of the samples. In the final step of sample preparation, we used DMSO to prepare stock solutions for both S1 and S2 because DMSO is known to be the universal solvent that it is able to dissolve most polar and non-polar compounds.