Reviewer's report

Title: Neurotrophic activity of standardized extract of Centella asiatica ECa233 on human neuroblastoma cells.

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Reviewer: A-Min Huang

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In this manuscript, Wanakhachornkrai and colleagues provided evidence to demonstrate that the standardized extract of Centella asiatica ECa233 have the neurotrophic activity comparable to BDNF. They used the human neuroblastoma IMR-32 as the cell model. Morphologically, they demonstrated that Eca233 promotes neurite outgrowth similar to the effect of BDNF. In terms of mechanisms, they demonstrated that ECa233 promotes neurite outgrowth through ERK1/2 and Akt signaling pathways. The manuscript is correctly written, however there are some points that need to be addressed to improve the quality of the manuscript.

1. What is the time point of the cell lysates in Figure 4? It was not mentioned when were the cells harvested after the treatment of Eca233? Since the neurite outgrowth assay is performed at 24 and 48 h, the increase of pERK and pAkt levels should be characterized in a time-dependent manner.

2. In Figure 5, 10 uM of LY and PD compounds already inhibit neurite outgrowth. It is possible that Eca233 promotes neurite outgrowth independent of ERK1/2 and Akt pathways. The authors should use a smaller concentration of these two compounds which themselves cannot inhibit neurite outgrowth but can decrease the enhancement of Eca233 to claim that it functions through these two pathways.

3. N and number of cells counted per condition and individual experiment in Figure 3 and 5B should be described in the figure legends. This is important to assess the appropriateness of data analysis and related statistical measurements.

Level of interest: An article of importance in its field

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