Reviewer's report

**Title:** Anti-Neuroinflammatory Effects Of The Extract Of Achillea fragrantissima

**Version:** 2  **Date:** 28 June 2011

**Reviewer:** Yong-Ki Park

Reviewer's report:

“Anti-neuroinflammatory effects of the extracts of Achillea fragrantissima” has been reviewed.

The authors demonstrated that the ethanol extract of Achillea fragrantissima is an effective anti-inflammatory agent that inhibits/attenuates the inflammatory responses in LPS-activated microglia. Primary microglia cultures are used to test its effects on the production of NO, and TNF-α, on expression levels of IL-1β, iNOS and COX-2. It is an interesting topic, but some things need improvement. However, the lack of in vivo results is the concern of this reviewer. It is not shown or stated in the text whether pinoresinol gets into the brain, or it has been used for the brain inflammatory and neurodegenerative disorders. There are several unclear parts and mistakes in the text.

Please see below for some comments:

Major Compulsory Revisions

1. Figure 1A. It is suggested to use another method to analyze cell viability and proliferation after treatment with Af extract and LPS, as 5-bromo-2-deoxyuridine (BrdU) and Trypan blue Assay, because the LDH test (alone is not appropriate). Also, it is commonly suggested to use cell viability after treatment with Af extract, as MTT or Trypan blue Assay. Furthermore, if authors used a commercial colorimetric assay kit (Roche Applied Science) for cell viability, it needs to more detailed description for it.

2. For the studies on the cellular mechanisms, activation of ERK1/2, p38 MAPK, JNK, cytosolic IκB and nuclear NF-κB p65 is necessary to measure by Western blot, EMSA or Oligo Pull Down in cytosol or nuclear extracts.

3. Measure also the activity of COX-2

4. Result 1, the authors explained that primary microglial cells were stimulated with different concentrations of LPS, but I could not find it in figure 1. Suggest the data of L-NMMA.

5. Figure 2. The result is a representative figure. Therefore, the results have to show histogram figure by a means ± SEM of 3 independent experiments (iNOS/#-actin or COX-2/#-actin). Authors used 100 µg dose Af extract only instead of various concentrations in this study. This should be discussed. It is commonly suggested to use 2 or 3 different concentrations of drugs for
anti-inflammatory efficacy tests, as ELISA, RT-PCR or Western blot. Figure 4 is the same as figure 2.

6. Why the authors studied the anti-neuroinflammatory activities of Af extract among many medicinal plants. Although Af extract showed a potent anti-inflammatory activity in microglial activation, its active components are unknown. Since similar effects of agents or medicines from plants had been reported in a huge number of studies, what are the advantages of Af extract that surpass others? This should be discussed. What this study will add to the existing knowledge of literature?

**Level of interest:** An article of limited interest

**Quality of written English:** Acceptable

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