Reviewer’s report

**Title:** Prokinetic and laxative effects of the crude methanolic extract of Viola betonicifolia whole plant

**Version:** 3 **Date:** 21 October 2012

**Reviewer:** Takio Kitazawa

**Reviewer’s report:**

Title of manuscript.
Prokinetic and laxative effects of the crude methanolic extract of Viola betonicifolia whole plant

In this paper, the authors examined the effects of the crude methanolic extract of Viola betonicifolia whole plant on intestinal transit, fecal output and smooth muscle contractility. The crude extract contained muscarinic receptor stimulant and caused prokinetic and laxative actions. These muscarinic actions partially contributed clinical use of this plant for indigestion and constipation. Followings were comments for improvement of manuscript. More experimental data are need.

1) Using invitro method, it is possible to evaluate the profile of muscarinic agonists. For example, the crude extract is separated several fractions using HPLC or other procedures. Muscarinic agonist might be contained but you cannot completely removed the possibility that the extract contains choline esterase inhibitors. Assay of cholinesterase activity is easy. Please try and check the possibility.

2) In vivo study was carried out using mice but the in vitro study was performed using rabbit and guinea-pig. Why the authors used different animal species in the experiments. Are there species-differences in the action of the crude extracts? It is better the authors add some in vitro data using mice GI tract.

3) The authors should indicate whether the crude extract contains atropine-resistant GI stimulants or not. Easy experiment way is as follow. 1, select concentration of CCh which causes the same amplitude of contraction with the extract. You should select the concentration of CCh which response was abolished by atropine. If the response to extract remains in the presence of atropine, non-muscarinic stimulant is contained in the extract.

**Minor comments**

1) The authors should indicate the name of experimental animals used in the manuscript title.

2) In vitro and in vivo is general. There is no hyphen.

3) Results, Please combine data of saponins and alkaloids as phytochemical analysis.
4) Please describe the behavior of mice after injection if there is no change.

5) Results. Effect of VBME on charcoal meal is not correct. Effect of VBME on charcoal meal intestinal transit is correct.

6) The authors should add the data about the effects of atropine itself on intestinal transit and fecal output.

7) Generally, in defecation study, the difference between wet weight and dry weight are measured for consideration of water content in the feces.

8) Please remove vertical lines in Table 1.

9) Fig. 1 is complicated. Please improve.

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

**Quality of written English:** Needs some language corrections before being published

**Statistical review:** Yes, but I do not feel adequately qualified to assess the statistics.

**Declaration of competing interests:**

I declare that I have no competing interests