

Review Form 1.6

Journal Name:	Journal of Pharmaceutical Research International
Manuscript Number:	Ms_JPRI_85433
Title of the Manuscript:	Inhibition of cell proliferation by Houttuynia cordata extract on gastric cancer cells via induction of apoptosis
Type of the Article	Short Research Article

General guideline for Peer Review process:

This journal's peer review policy states that **NO** manuscript should be rejected only on the basis of '**lack of Novelty**', provided the manuscript is scientifically robust and technically sound. To know the complete guideline for Peer Review process, reviewers are requested to visit this link:

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PART 1: Review Comments

	Reviewer's comment	Author's comment (if agreed with reviewer, correct the manuscript and highlight that part in the manuscript. It is mandatory that authors should write his/her feedback here)
Compulsory REVISION comments	<p>Major suggestions</p> <p>1) Scratch migration assay- its preferable to include results of 48 hours and 72 hours and the area measured by an appropriate software like ImageJ</p> <p>2) Cell migration assay- standard protocol preferred</p> <p>3) Fold expression change in real time data is</p> <p>4) Real time PCR and data analysis details in methodology.</p> <p>Minor suggestion</p> <p>1) Lack of details regarding cell line</p> <p>2) No magnifications mentioned in MTT</p> <p>Decision</p> <p>Acceptable after major revisions</p>	<p>Dear Reviewer, thank you very much for your valuable comments on our manuscript. The revised section was shown below:</p> <p>❖ Major suggestions</p> <p>1) Scratch migration assay- its preferable to include results of 48 hours and 72 hours and the area measured by an appropriate software like ImageJ: - We totally agree that it is preferable to include results of 48 hours and 72 hours in scratch migration assay. The fact that the inhibition of the extract on cell migration during 48h and 72 h was not significant at the concentration of 50 µg/ml. Therefore, we try to increase the treatment concentraton of the extract up to 100 µg/ml. In this case, the extract exhibit significant inhibition on cell migration up to 72h. However, this inhibition was due to induction of cell death that is not preferable for migration assay. Thus, we have decided to show the inhibitory effect of extract on cell migation up to 24h. - Moreover, the relative gap area has also measured by ImageJ according your kind suggestion ➔ Page 5, lines 84-86</p> <p>➔ Page 9&10, lines 153-159</p> <p>2) Cell migration assay was followed the protocol as described by Kwak and Ju [15] and the relative gap area has also measured by ImageJ (Kwak Y, Ju J. Inhibitory activities of Perilla frutescens britton leaf extract against the growth, migration, and adhesion of human cancer cells. Nutr Res Pract. 2015;9(1):11-6.)</p> <p>3) Fold expression change in real time data is the expression level of target gene that was calculated by following formula below : - The expression level of genes was calculated as following formula (1) and (2): $\Delta Cq = Cq (Tar) - Cq (Ref)$ (1) $\Delta\Delta Cq = \Delta Cq (Exp) - \Delta Cq (Con)$ (2) Where, Cq = quantification cycle; Tar = Target gene; Ref = Reference gene (GAPDH); Exp = Experimental; Con = Control - Moreover, the Figure 3 have been corrected as shown in Page 11, lines 180-182</p> <p>4) Real time PCR and data analysis details in methodology We have added the data analysis details in the Real time PCR method ➔ Page 6, lines 104-109</p> <p>❖ Minor suggestions</p> <p>1) Lack of details regarding cell line: ➔ Page 4, lines 59-61</p> <p>2) No magnifications mentioned in MTT ➔ Page 5, lines 78-79</p>
Minor REVISION comments		
Optional/General comments		

PART 2:

	Reviewer's comment	Author's comment (if agreed with reviewer, correct the manuscript and highlight that part in the manuscript. It is mandatory that authors should write his/her feedback here)
Are there ethical issues in this manuscript?	<u>(If yes, Kindly please write down the ethical issues here in details)</u>	