Reviewer’s report

Title: Validation and Quantification of major biomarkers in ‘Mahasudarshan Churna’- an ayurvedic polyherbal formulation through high-performance thin-layer chromatography

Version: 1 Date: 17 Apr 2020

Reviewer: Bronislav Jurásek

Reviewer's report:

I would like to thank the authors for including the HPLC analysis in the supplementary material, however, I have few comments on that.

* The authors should include their conditions (concentration of standards, samples, column, …).

* What are the numbers in the chromatogram 2b?

* What is the purity of the Magniferin standard? It looks on the HPLC, that there might be several impurities inside.

* If the analyses (chromatogram 1b and 2b) have the same conditions, there are several peaks at 4.0-6.0 min in the chromatogram 2b which are missing in the 1b chromatogram in the 4.0-6.0 min region.

* The chromatograms show that the identification may work correctly. However, could you please do also the quantification of the compounds in the samples by HPLC? HPTLC is a novel technique (especially for quantification in this field), which may have significant value for analytical centers dealing with the analyses of such medicine, but without the proper comparison, how precise is this method, it loses its value. I highly recommend comparing the results with the HPLC technique.

Few suggestions for the manuscript:

The authors revised the manuscript. However, in the manuscript remained several typos (for example: page 3, line 25-26: used for to cure, page 4, line 45-46 Curcma longa L., page 5 divide the first sentence (HPTLC system …) into 2 sentences, page 11, line 22, Curcma)

In the revised manuscript, the figures look better. It would be even better if you could render them in the same style (size of the digit fonts). It was nearly impossible to read for example the numbers in figure 4.
As the last suggestion, as you used 3 solvent mixtures it is a little bit difficult to find which solvent mixtures were used for which compounds. It would be worth to insert that either in table 1 or in the caption of figure 4. That would make that more clear.

I believe, that if you do the quantification with HPLC as an evidence, that the HPTLC technique is precise enough to compete with the HPLC, it would dramatically enhance the quality of the manuscript.

**Are the methods appropriate and well described?**
If not, please specify what is required in your comments to the authors.

Yes

**Does the work include the necessary controls?**
If not, please specify which controls are required in your comments to the authors.

No

**Are the conclusions drawn adequately supported by the data shown?**
If not, please explain in your comments to the authors.

No

**Are you able to assess any statistics in the manuscript or would you recommend an additional statistical review?**
If an additional statistical review is recommended, please specify what aspects require further assessment in your comments to the editors.

Not relevant to this manuscript

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Please indicate the quality of language in the manuscript:

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