Reviewer's report

Title: The use of Euphorbia hirta L. (Euphorbiaceae) in diarrhea and constipation involves calcium antagonism and cholinergic mechanisms

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Reviewer: Ephrem Engidawork

Reviewer's report:

I thank the authors for providing a detailed response for the queries I raised. I have, however, some concerns that the authors should address before reaching a decision on the manuscript.

* The effort made to revisit the rationale for conducting this study is laudable. However, there is still a need for attending language issues.

* You could modify the title as "The use of Euphorbia hirta L. (Euphorbiaceae) in diarrhea and constipation involves calcium antagonism and cholinergic mechanisms"

* Page 11, Preparation and extraction: plant collection and identification as well as specimen deposition should be included.

* I am not still convinced about the fractionation. The hydroalcholoic crude extract (70% methanol) was used for fractionation with solvents of differing polarity. The question here is what's the likelihood of getting constituents soluble in non-polar solvents (Pet. Ether) from a semi-polar-to polar crude extract?

* Laxative effect higher in physiological than pathological conditions (lopermiade-induced constipation), indicating diarrhea is a side effect rather than a therapeutic effect. What do the authors say about this assertion? Table 3, once you compare loperamide treated group with controls, you need to compare loperamide+extract group with loperamide only treated group to show the ability of the extract to reverse loperamide-induced constipation. Loperamide+extract group can also be compared with normal controls to see if the extract is able to return bowel movement to the basal level.

* Rabbit jejunum (Page 16, Line 41-51): The authors alluded that the chloroform, ethylacetate and aqueous fractions inhibited the spontaneous contractions and their effects were potentiated in the presence of atropine. However, the inhibitory effect of pet-ether fraction was remained unchanged when reproduced in the presence of atropine (Fig. 4). Similar to parent extract, the fractions of E. hirta also equally inhibited the high and low K+-induced contractions except aqueous fraction, which showed partial inhibitory effects (Fig. 4). I would say the chloroform fraction also behaved like the pet-ether fraction if one sees Figure 4, although the EC50 value given in Table 3 appeared to be a little bit different. In addition, no apparent change could be discerned among the crude extract and fractions in their effect in the presence of high and low K+ concentration, except the
aqueous fraction, where the inhibitory effect was mild. I think the statement "The extract and fractions of E. hirta equally inhibited the high and low K+-induced contractions" is misleading. If one looks at Figure 3 & 4, the inhibition obtained at high and low concentration did not differ. Since high K+ is associated with Ca2+ influx and low K+ with K+ channel activation, the lack of difference between the two strongly suggests that Ca2+ antagonism rather than K+ out-flux to be the likely mechanism. Discussion should be rewritten in such a manner.

* Table 1 and 2: delete 10 from the dose column for the control and replace it with "-".

* Cite Tables consecutively in the text. For example, Table 3 is a new addition and cited in the text as EC50 values.

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.

Yes

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

Yes

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I am able to assess the statistics

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