Author’s response to reviews

Title: Defatting of acetone leaf extract of Acacia karroo (Hayne) enhances its hypoglycaemic potential

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Reviewer reports:

Ephrem Engidawork, PhD (Reviewer 1): * This is an interesting study that attempted to examine the hypoglycemic effect of an extract from enzyme inhibition to downstream signaling pathways. However, there are some inconsistencies that should be explained.

* Qualitatively defatting of the plant material did not influence the presence of secondary metabolites. However, quantitative studies showed that defatting led to loss of phenolic compounds, although the authors did not say whether this loss was statistically significant.

Response

The loss of phenolic compounds was not statistically significant and has been indicated (corrected) in the manuscript
* Following defatting, solvent mixtures appear to yield more secondary metabolites with antioxidant potential than single solvent. It would have been better had the authors used a combination of solvents.

Response

Thanks for the comment. Since it is the first time this plant is being reported to have hypoglycemic potential, we are sure that this comment will come in handy on further exploration of hypoglycemic potential of this plant.

* The authors used two methods for evaluating antioxidant capacity and defatting produced opposite effect in the two methods, a decrease in DPPH and an increased activity in the iron reducing assay (here also nothing was said about the statistical significance of the difference). The two assays usually tend to provide a similar trend, as antioxidant capacity in both methods involves electron-donating mechanisms. It would have been good had flavonoid content determination was done, as they also significantly contribute to antioxidant

Response

The statistical differences have been included and the explanations on the two assays have been thoroughly revised.

Flavonoids could have given a good explanation, however, the opposite cannot be ruled out. This also applies to other phenolic compounds such as tannins, but this also cannot be guaranteed. We have thus added a suggestion in the manuscript that taking into account the flavonoids and tannin contents might also explain why the outcome is as observed.

If in any case flavonoids have to be taken into account, because of seasonal variation that influences the phytochemical outcomes, the plant material can only be collected in the month of March.

* Defatting produced better α-amylase inhibition than non-defatted extract and polyphenols were implicated for the difference, although defatting tended to decrease polyphenols. Flavonoid content might have provided better explanation.
Response

There is a possibility that flavonoids could give a better explanation of the outcome observed. This could also be acceptable in any case of any phenolic compounds provided they have such inhibitory activities. Because the active ingredient (compound of interest) is unknown, we carefully attributed our finding to phenolics, with a possibility of antioxidants such as ferric reducing power contributing to the outcome observed. Furthermore, the defatting process may have removed interfering compounds or unmask the potential with a direct bearing on the phenolic outcome.

* Glucose uptake did not vary with defatting in the two concentrations used in muscle cell lines. However, variation was observed with the lower concentration in adipocytes, but not with the higher concentration. What could be the source of this variation?

Response

We are not certain of the source of the variation between the two concentrations in the adipose cells. But since we are dealing with crude extracts unlike pure compounds, it was best for us to suggest that different cell lines respond differently to different compounds, in which case, a possibility of interfering compounds from the non-defatted extract as observed.

* Extract and insulin combination treatment produced an inconsistent and at times antagonistic effect, casting doubt on the potential use of the extract for treatment of diabetes. This seemingly antagonistic activity was not observed only in translocation but on kinases determined in the study. I think this issue should be looked at seriously and the authors need to come up with an acceptable explanation. Moreover, it would be good if the authors show the link between the kinases determined and insulin signaling pathways or blood glucose regulation.

Response

Since we are dealing with the crude extracts in the study, we indicated that the extract might have compounds that interfere with insulin response as observed. Nonetheless the potential of the extract alone indicates that its is an insulin mimetic, although it does not necessarily act through the primary insulin target (Akt), it indicates that it acts downstream. This case however stands out to be more beneficial among individuals with absolute absence of insulin and most severe cases of insulin resistance; bearing in mind that the extract has the possibility of targeting proteins downstream Akt resulting to glut 4 translocation, in which case insulin cannot (in severe
insulin resistance). We have also indicated that despite the plant having these effects (particularly the antagonistic effects) isolation of the active compounds could perhaps tell what could be the main cause of antagonism, whether the active compound or its just interfering compounds. Also, in vivo studies would help clearly elucidate the potential of the plant as an antidiabetic remedy. Most importantly, so far, the plant promises to have the leads against diabetes.

George Awuku Asare (Reviewer 2):

*Re-examine statistics. It is more appropriate to use Mean +/-SEM rather than Mean +/-SD

Response

The statistics has been thoroughly examined and the results have been represented as Mean +/-SEM rather than Mean +/-SD.

*Minor comment in abstract; Colorimetrically and not calorimetrically

Response

Thanks. The mistake has been rectified in the manuscript.