



SDI FINAL EVALUATION FORM 1.1

PART 1:

Journal Name:	<u>International Journal of Biochemistry Research & Review</u>
Manuscript Number:	Ms_IJBCRR_26369
Title of the Manuscript:	The inhibitory potentials of seed kernel methanol extract of Mangifera indica L. anacardiaceae and fractions on metalloprotease-induced toxic effect from Echis ocellatus venom
Neew title:	The inhibitory potentials of seed kernel methanol crude extract and fractions of Mangifera indica L. Anacardiaceae on metalloprotease-induced toxic effect of <i>Echis ocellatus</i> venom.
Type of Article:	Original Research Article

PART 2:

FINAL EVALUATOR'S comments on revised paper (if any)	Authors' response to final evaluator's comments
Please correct as follows :- <u>Materials and Methods</u> Line 51: was protein concentration determined for the venom collected? Normally protein determination used to measure the amount of venom instead of weighing them. Line 53: What is the voucher number once specimen deposited in a herbarium? Line 57: methanol extraction can lead to extraction of tannins and tannins (polyphenols) been known to non-specifically bind to proteins or other macromolecules. As venom known to contain majority of lethal proteins the neutralization can be due to this non-specific binding and neutralization of the venom. Suggested to review this non-specific binding and include within the discussion if the results may been due to tannins rather than specific inhibitory effects on the metalloprotease present. Line 61: How specifically the researchers focussed on the metalloprotease present in the venom without performing any protein purification technique? Line 65 and Line 75 and Line 80: These three segments provided impressions the experimental animals used of confusion. Reviewer suspects the actual animal used was rats as Wistar normally related to rats. In line 75, reviewer surprised if a mouse was used it is unlikely to provide 2 ml of blood and lastly, in line 80, researcher wrote rats instead of mice. Please be certain of the animal used and write accordingly. Line 78-83 and Line 84-90: Reviewer is concerned the techniques used on the experimental animals are not acceptable currently if to be approved for animal ethics by any IACUC. It is therefore assumed the researcher did not obtain any animal ethics approval (as none being mentioned within the text). The reviewer therefore left it to the journal management decision if still acceptable if no animal ethics approval was ever been sort before the research work been conducted. The reviewer therefore request the researcher to identify one latest journal publication still employing such techniques and being approved for animal ethics?	Protein determination was carried out for the crude venom and partially purified venom. The biuret test voucher number: 1312 (Metalloprotease specific assay was carried out using casein as substrate to determine inhibition of activities after each purification step thus metalloprotease inhibition was inferred. This does not mean that the researchers are not aware of other means of inhibition of the snake venom components) This venom was partially purified on a two-step purification process using ion exchange chromatography on DEAE sephadex and gel filtration on sephadex G-75. (I have attached a the purification table at the end of the manucrypt) The actual animal used is albino mice and not rats. (Yes more than 80% of the mice yielded up to 2ml blood and those that were not up to 2ml were enough for the analysis) The mistake in line 80 has been corrected thanks. Animal ethics approval was not obtained from any IACUC but the experiment was carried out at the animal house of the department of pharmacognosy, Ahmadu Bello University Zaria with a strict guidelines on animal ethics.



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Line 92: Reviewer request the researcher provide reference this technique been taken from due to the unorthodox method described.	National Committee for Clinical Standards. Reference and selected procedures for the quantitative determination of hemoglobin blood, 2 nd ed. H15-A2. Villanova, Pa.: NCCLS, 1994.
Line 100: the statement....."5ml of reagent...." What is "reagent" here?	cyanmethaemoglobin (Drabkin's) reagent
Line 102:"using standards".....what standards used?	cyanmethaemoglobin standard
Line 103: provide reference for this technique taken from?	Warrell DA, Davidson NMCD, Greenwood BM, Ormerod LD, Pope HM, Watkins BJ, <i>et al.</i> , Poisoning by Bites of the Saw-Scaled or Carpet Viper (<i>Echis carinatus</i>) in Nigeria. <i>Quarterly Journal of Internal Medicine XLVI</i> . 1977, (181): 33-62.
Line 109: Explain how total protein can be obtained accurately using refractometer? Normally a dye such as Bradford and a standard curve used to obtain protein measurements.	The refractometer is a device used to measure the refractive index of a solution. Refractive index is defined as the degree that the light is bent when it is passed through a liquid, and is a function of the amount and type of solid material dissolved in the liquid. In clinical practice, this instrument is calibrated to give direct readings of total protein in serum samples.
Line 114: need more information how the researcher calculated the "activity" (micro mol/min)? and also the % inhibition i.e for example 58% is based on what control? Also explain how the methanol extract provided lower % inhibition compared to the ethyl acetate if the latter been extracted from the former? Has all extracts been quantitated to be same concentration used throughout this experiment?	(Enzyme activity was determined from the slope of the line from the graph of absorbance vs. time and correlating the result with standard curve of tyrosin. Percentage inhibition was calculated using the formular; % inhibition = [(normal activity - inhibited activity) / (normal activity)] * 100%) with normal activity as the control without inhibition. The ethyl acetate gave the more inhibition than methanol probably because during the fractionation of the methanol extract, ethyl acetate was able to isolate or expose a component which activity was previously subdued in the methanol extract.
Line 119: The table is based on observation that is not done with a proper rubrics i.e if there is swelling the researcher is requested to provide degree of swelling for e.g – no swelling; + mild swelling; ++ swelling ; +++ severe swelling for better classifications. Do the same for all other observations.	This has been reviewed on the table
Line 132 till 136: in both Table 3 and Table 4, the use of "low" and "high" not encouraged for a scientific study as not measurable. Reviewer request researcher determines LD50 or MLD of venom and use instead.	
Discussion	
Line 162: The researcher to make a statement the metalloprotease been inhibit may not be accurate unless the total metalloprotease been firstly fractionated or performed a very specific experiment related to the metalloprotease itself?	This has been reviewed in the place where it was first mentioned in line 62 above.
Line 177-185: reconsider to include until a proper action been done for the observations as suggested earlier or else this outcome not very relevant to conclude.	This has also been reviewed above
Ethical issue: One is the animal ethics approval by a proper IACUC.	