



SDI Review Form 1.5

PART A:

Journal Name:	International Journal of TROPICAL DISEASE & Health
Manuscript Number:	MS: 2012/IJTDH/1693
Title of the Manuscript:	Anti-diabetic activity of aqueous extracts of <i>Vitexdoniana</i> leaves and <i>Cinchona calisaya</i> bark in alloxan – induced diabetic rats.

(Note: Title of different sections as proposed below may differ in case of review paper)



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PART B: Review Comments

	Reviewer's comment	Author's comment (if agreed with reviewer, correct the manuscript and highlight that part and write here 'Corrected'/ if not agreed, give suitable justifications)
General comment:		
Specific comments:	See below	
Title and abstract	The title and abstract are suitable for the article	
Introduction (Is the problem/objective of this study original, important and well defined?)	The objective of this study is well defined. The originality of the paper, as argued by the authors, is given due to the lack of scientific data on the use of the 2 plants for the treatment of diabetes. The main focus of the article, however, is on the anti-oxidative properties of both plants. However, it is already known in the literature that <i>V. doniana</i> and <i>C. calisaya</i> have anti-oxidative properties (Biochem Res Int. 2011 Agbafor KN & Nwachukwu N.) (Journal of Pharmacognosy and Phytotherapy Vol. 4(4), pp. 44-52, July 2012 Antifungal, antibacterial and antioxidant properties of Adansonia digitata and Vitex doniana LAGNIKA et al). The phytochemical analysis done in this paper does not give precise information. There is already scientific research done and phyto-compounds are published for these plants.	I think that these analyses are still necessary since the influence of the environment on the phytochemical composition and biological activity of medicinal plants cannot be over emphasized.
Are all the references cited relevant, adequate? Are there any other suitable current references authors need to cite?	References cited are relevant. However there are some discrepancies in the referencing between the in-text reference and the reference list - e.g. Baren(n)ess (Line 49). Also references used in-text are not in the reference list - e.g. Aebi (Line 119). There are also references in-text where a space is missing between the name of the author and et al. (Line 35, 44) or wrong punctuations are used (Line 58, 248).	Corrected



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Materials & methods (Kindly comment on the suitability of the methods. Sufficient details of the methods should be provided to allow peers to evaluate and/or replicate the work)	2.2 Experimental animals: Has ethical approval been obtained and the principals of animal care been followed? The Wistar rats that were used for this experiment have a huge variation in body weight (100g – 175g). Why is that? It is a concern for the outcome of the study as a higher body weight or older age of the rat may be a factor that leads to diabetes (type 2) itself. 2.4. Preparation of extract: Is the preparation method for this experiment similar to what the traditional preparation would be? A concern is the evaporation of 2 liters of water at 80 degree and storage of the crude extract in the fridge (4 degree celcius?). Will this not affect the quality of the extract? 2.5. Determination of effects There is no mention in the method section of how the extracts are administered in the animals, although the abstract mentions an intra-peritoneal administration. This way of administration is difficult to compare to a traditional use, where the digestive system will alter and metabolise the plant compounds before they act on the body cells. Alloxan was used to induce diabetes. Alloxan is toxic to beta cells and therefore will induce Type1 diabetes by destroying the insulin producing cells. However the control drug used was Glibenclamide, which is a drug used to only treat diabetes type 2 (as it stimulates the beta cells to release more insulin). Was the food intake of the rats monitored? There is a possibility that the intra-peritoneal injection of plant extract or control drug may have caused nausea or stress in the rats and the blood glucose decreased due to low food intake. Ideally there should have been a another diabetic control group that was injected with water to give the same stress level to the rats.	Yes, ethical approval was obtained from the committee and all the rules guiding animal care followed. Rats of moderate sizes that could withstand the effect of alloxan were selected while rats weighing 200-250g were rejected. The rats were grouped according to there weights and all theadministrations were done according to the weights of the animals.The rats were confirmed non-diabetic before the experiment. 2.4 Yes, extraction was carried out to reflect the traditional method. Volume of extract obtained after macerating for 18h with 2L of water was far below 2L. The extract was refrigerated to prevent microbial contamination and this did not affect the activity. 2.5 Corrected I think both routes belong to the same enteral routes, although the effect of the digestive enzymes is more when given orally. The mechanism of action of alloxan is not yet clear. It also interferes with early-phase insulin responses thereby simulating the insulin secretory defect observed in Type 2 diabetes. It also damages glucose receptors as seen in Type 2. Food intake was not reduced, rather polyphagia(enhanced appetite) was observed.
Results & discussion (Kindly comment on: 1. Are the data well controlled and robust? 2. Authors should provide relevant references during discussion. 3. Discussion and conclusions should be based on actual facts and figures. Biased claims should be pointed out. 4. Are statistical analyses must for this paper? If yes, have sufficient and appropriate statistical analyses been carried out?)	Section 3.1: no P-values are reported at all Figure 1: no data for Alloxan group is reported, no P-values are reported at all Figure 2 & 3 & 4: no P-values are reported Section 3.5 and Table 1: add little value to the paper as there is already published information for phytochemicals from both plants. Discussion: The authors give good discussion and reasoning for the anti-oxidative activity of both plant extracts. However, the discussion in the first paragraph regarding the anti-diabetic action leaves open questions. The reviewer disagrees with the statement that the	Corrected Corrected The P-values have been reported in sections 3.1 – 3.4 Corrected



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	plant extracts give comparable results to the standard anti-diabetic drug. The authors themselves state otherwise in section 3.1. Also, there is no explanation why C calisaya is more potent at the lower dose.	C. calisaya extract may be exhibiting a saturation kinetics whereby the activity remains the same or decreases when all the receptors are fully occupied. This is yet to be investigated.
Are there any ethical or competing interest issues?	No	
Conclusion (Is the conclusion supported by the data, discussed inside the manuscript? Conclusions should not be biased and should be based on the data, presented inside the manuscript only)	As mentioned by the reviewer earlier 9methods section) – it is questionable that the anti-diabetic effects where caused by the plant extracts. The experimental setup and control groups (and control drugs), need to be reconsidered to give robust data.	The antidiabetic effects were caused by the extracts since both negative and positive control groups were employed in the experiment and there were remarkable differences between the extract- treated groups and the controls.
References	See reviewers comments above for the referencing.	Corrected