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Journal Name:	International Journal of Biochemistry Research & Review
Manuscript Number:	2014_IJBcRR_8986
Title of the Manuscript:	PARTIAL PURIFICATION AND CHARACTERIZATION OF EXTRACELLULAR PROTEASE OF <i>Shigella dysenteriae</i> AND ITS INHIBITION BY THE VOLATILE OIL OF <i>Thaumatococcus danielli</i>
Type of the Article	Original Research Article

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This journal's peer review policy states that **NO** manuscript should be rejected only on the basis of '**lack of Novelty**', provided the manuscript is scientifically robust and technically sound.

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PART 1: Review Comments

	Reviewer's comment	Author's comment (if agreed with reviewer, correct the manuscript and highlight that part in the manuscript. It is mandatory that authors should write his/her feedback here)
Compulsory REVISION comments	Please check carefully the language along all the manuscript. ABSTRACT: - Line 2. Please inform here the "selected enteric pathogens" and replace "the partially" by "a partially". - Please rewrite the sub-section "Study design". As written it seems a list of unconnected words. - Also rewrite "Place and Duration of Study". - The protease was not purified by "dialysis and gel filtration" but by "ammonium sulphate salting-out followed by gel filtration chromatography". - Rewrite "Enzyme assay with casein and volatile oil as substrate and inhibitor respectively". There are no verbs in this sentence. Rewrite. - "Highest purification fold (...)" was obtained after what step? - What means "a peak for total protein"?	Done Corrected Corrected Corrected Corrected Corrected Corrected (after Sephadex G-75 gel filtration) The sentence has been expunged. We actually wanted to communicate that the chromatogram showed a peak for total protein and a peak for enzyme activity.



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	- The results in this paper provide no basis for state that "this plant will be a promising natural product with pharmaceutical effects". INTRODUCTION Lines 16-18. These first sentences are vague and dispensable. Line 20: "as source of drugs". I believe that it is better to state that people use plant extracts "to treat diseases". Line 24. I did not understand the expression "pathogenic/worm". Worms are also pathogenic and are not the unique pathogens target of pharmaceuticals. Line 26. Replace "plants" by "plant" and "Africa" by "African". Line 33. Please explain why osmotin activity was "selective" or delete this word. Line 35-36. "Anthony et al. [11] (...) properties". Please rewrite. These authors evaluated "some chemical components" or "determined the chemical composition of the oil"? Lines 42-43. "Released toxins" are not the same of "membrane-located toxins". Please revise. Line 46. Delete "amongst other species". Authors should comment more in Introduction why they selected enteric pathogenic bacteria.	We reviewed this suggestion and considered it appropriate. The statement has been redacted. The statements have been reviewed and re-drafted. We consensually agreed with this. Worm has been deleted for clearer meaning Corrected Deleted This has been carefully read and corrected Corrected Done Carefully corrected
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	Line 60-63. "Previously (...) food spoilers". Please rewrite these sentences in order to evidence the purpose of including them in the text. As written, they appear to be dispensable. Line 63. Replace "this work is" by "this work was". Line 64-65. Replace "its extracellular protease" by "extracellular proteases". MATERIALS AND METHODS Line 74. Was the identification really performed? Is there any voucher specimen number? Also, are you sure that the identification was performed using the foliage? Lines 91-95. Revise language. Line 103. And how were prepared the cultures used in the antibacterial assays? How long the cultures were incubated? What was the population density used? There are many details lacking in description of antimicrobial assay. Line 121. Delete "susceptible" and rewrite the sentence. As written it seems that the medium is susceptible to anything. Line 122. "21.5 x 17 cm²" or "21.5 cm x 17 cm"? Lines 123-127. Rewrite all this. It is not clear how was determined MIC and MBC. Were MIC aliquots transferred to agar plates to determined MBC? If yes, this is not described here.	Corrected Corrected The entire sentence has been redacted Foliage literally leaves. The leaves of the plant was identified and authenticated but not vouched with any code. Done Preparation of the culture is universal and usually written on the pack of the medium. Other suggestions have been taken care of. Done Done Corrected
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	Delete "favourable conditions". This is subjective and dispensable. Line 129. Replace by "Crude enzyme extract from <i>S. dysenteriae</i>". Line 133-134. Please specify the "room temperature". Lines 142-146. It is dispensable to describe Lowry's method. Line 147. Was BSA standard used only at this concentration? Or was performed a standard curve using several concentrations? Line 159. Replace "A unit" by "One unit". Line 162. Replace "Dialysis" by "Salting-out". Line 164. How was obtained the "saturated solution"? Was ammonium sulphate previously homogenized by a determined period before dialysis? And centrifugation was performed after dialysis? Also, dialyzed against what? Revise this item to make clearer this methodology. It is very confusing.	Done Previous reviewer has offered similar suggestion, which has been done Done Describing Lowry's method may not be considered dispensable because it portrayed the way we understood the method and how we carried out. Moreover, it will make easier for reproducibility. This was done using several concentrations to construct standard graph. The concentrations have been stated in the manuscript. Done Corrected We already prepared a standard saturated solution of ammonium salt. The desired saturation was then prepared using the extract as solution, which was added to calculated volume of previously prepared saturated solution. The mixture in the dialysis tube was dialyzed for 48 hours at 20°C. At the end of this dialysis, the solution was centrifuged and the residual supernatant was again used for 55% and 65% sequentially.
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	Line 173. "This was carried (...) for 72 hours" is dispensable. Line 175. Rewrite "to separate the 65% (NH₄)₂SO₄ dialysate". This dialysate corresponds to precipitated or supernatant proteins. Line 176. Gel filtration chromatography does not use any eluent. So, replace "50 elutions" by "50 fractions". Replace "elution contained 3.0 mL" by "fraction contained 3.0 mL". Delete "of eluent and for each the eluent; both total (...) were carried out" and replace by "Each fraction was evaluated for total protein and enzyme activity". Line 177. Replace "Eluents" by "Fractions". Line 197. "partially purified enzyme" corresponds to what? What are the fractions pooled after gel filtration? Delete "extract". Lines 201-202. Rewrite "Protease assay (...) an inhibitor". RESULTS AND DISCUSSION Lines 207-209. Rewrite. Line 210. Start the sentence by "The results of". Line 220. Delete "under favourable conditions". Revise the Figure 1. Total protein was determined by OD250 nm or Lowry's method? Also, I believe that in chromatogram it is showed the absorbance at 250 nm and not the total protein. In X-axis, delete "Eluted". In	Done Corrected The same has been earlier cited by previous reviewer and it has been corrected. Corrected Pooled fractions; Fractions 10 – 13 for protease activity and 11 – 17 for total protein respectively. Extract has been deleted. Corrected Redacted Done Done The Lowry's method was used to determine total protein and the colour solution was read at 750nm and not 250nm, likewise the Folin Ciocalteau's method used for enzyme
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	caption, please inform the number of fractions pooled and called as “partially purified enzyme”. Why authors did not perform at least a PAGE and SDS-PAGE of sample to show how many proteins are present in the preparation obtained after gel filtration column. Remember that the authors started the title emphasizing the enzyme purification and a PAGE and SDS-PAGE profile is at least “mandatory”. Please comment more how an essential oil could be act as a competitive inhibitor of a protease? What interactions can be occurring between the oil and active site? Line 321. Replace “speed” by “velocity”. Also, define what means $\Delta A/\text{min}$. It is not possible to see the entire Lineweaver-Burke plot since half of the figure is out of the page. Please discuss on the implication of the inhibition of extracellular protease for bacteria survival. Lines 353-355. Rewrite or delete to avoid redundancy in regard to introduction. Also, why mention “cosmetic” purposes? Lines 357-359. Strongly rewrite and revise this sentence. The fact that the oil inhibited protease does not imply that “antibiotics do not only inhibit beta-lactamase (...)”. This result only means that your oil inhibited the protease but does not inform anything about a general mechanism for “antibiotics”. Also, remain in mind that beta-lactamase inhibitors are only a class of antibiotics and there are several	activity. Both processes used availability of tyrosine and other aromatic amino acids as indexes for spectrophotometric quantification. Yes, but we are financially incapacitated as most of these works were solely run on our personal expenses. In our future work, we will incorporate these techniques. This has been done Done Realigned The implication has been incorporated into the manuscript. This has been totally re-written. The entire sentence has been re-discussed.
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	other mechanisms described for many other antibiotic classes. Is there no information on the composition of the oil studied in this work? This will improve significantly the study impact. Line 369. "Most of the proteins released were enzymes". How you affirm this? There is no characterization of homogeneity and evaluation if other proteins are present in the sample. Line 370. "Enzyme with catalytic effects" is a redundant expression. Lines 371-372. "The protein is unstable at optimal conditions" and "alteration of optimal conditions reduces catalytic effects" are distinct aspects. Revise this discussion. Line 377. "of the same enzyme". Please replace by "of protease preparation".	Ojekale et al, 2013 have determined the volatile constituents of the essential oil of this plant. Nevertheless, we can still run it in our subsequent study using different approach and technique. Thank you for this observation. The choice of our microbial medium accepted the release of many enzymatic proteins but remember that enzymes are specific. So, in the mist of hundreds of proteins, specific substrate still attracts specific enzyme. The use of casein is hypothetically assumed to attract proteases. However, our report does not offer characterization of homogeneity of the studied enzyme but the adopted method instructed us that we were majorly working on proteases. Nevertheless, we will try as much as possible to incorporate this into our subsequent study. Done Done Done
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	Lines 382-383. There is no result that supports the speculation that the protease is a “dimeric protein”. Delete or explain it. CONCLUSION. Lines 397-398. Rewrite or delete.	This statement was unintentional placed. It has been deleted. Done
Minor REVISION comments	This work needs a strong language editing. Also, authors should reformulate the discussion in order to reducing the amount of informations already mentioned in introduction and focusing more on discussion of their own results. There are also some mistakes and sentences that need to be cleared as listed above.	These, and every other suggestion, have been revised and deemed amendable. All the sentences have been redacted. The discussion has been totally overhauled and edited. Thank you.
Optional/General comments		