



SDI FINAL EVALUATION FORM 1.1

PART 1:

Journal Name:	International Journal of Biochemistry Research & Review
Manuscript Number:	MS: 2012/IJBCRR/2438
Title of the Manuscript:	Antifungal activity of Terminalia mantaly on the in vitro growth of Cryptococcus neoformans

PART 2:

FINAL EVALUATOR'S comments on revised paper (if any)	Authors' response to final evaluator's comments
The authors apparently do not take into consideration the inaccuracy of counting cells to get to a value of 1000 cells for different repetitions. It is impossible to compare their results to that of other publications in leading journals in this field. The question to ask is what was the MIC in mg/ml and how this value compares to that of the positive control (a standard antifungal reagent)?. They have not included a positive control. If you have a reduction of 80% in the number of colonies it may be irrelevant in a physiological system because three doublings would attain the original concentration. They do not have to lecture me on the importance of Cryptococcus neoformans. We have screened leaf extracts of close to 700 trees for activity against this organism. The methods they used make it impossible to compare the activity of the plant extract against this pathogen with the results of other authors. <i>The following references may help them to improve the work.</i> MOKOKA TA, Mc GAW LJ, ELOFF, JN (2010) Antifungal efficacy of ten selected South African plant species against <i>Cryptococcus neoformans</i> Pharmaceutical Biology 48, 397–404 MASOKO P, J PICARD and J.N ELOFF [2005] Antifungal activities of six South African <i>Terminalia</i> species (Combretaceae) Journal of Ethnopharmacology 99:301-308 MASOKO P and J N ELOFF [2005] The diversity of antifungal compounds of six South African <i>Terminalia</i> species (Combretaceae) determined by bioautography. African Journal of Biotechnology 4, 1425-1431 The methods used are described in more details in ELOFF J N 1998 A sensitive and quick microplate method to determine the minimal inhibitory concentration of plant extracts for bacteria. Planta Medica 64, 711-714	The determination method of the colonies number is a classic method generally used in microbiology. To check the charge of the inoculum, secondary dilutions of the inoculum was performed (10^{-2}, 10^{-3}, 10^{-4} and 10^{-5}). All these suspensions were seeded on agar in Petri dishes incubated at the same time as the test cultures. After 48 hours of incubation, the colony count of 10^{-5} dilution, based on the sampled volume (10µL) and the dilution factor, give an inoculum charge, which is consistent with normal standard for culture. In this verification, the number of colonies counted in suspensions (10^{-4}, 10^{-3}, 10^{-2}, 10^{-1}) are taken into consideration. From this method we obtain some very isolated colonies and their reckoning can be easily done (less colonies to count with a pen colony counter). The rest is but a reckoning report. We can't have a lack of precision reckoning report. We can't have a lack of precision reckoning. We don't directly count the 1,000 cells. (We have included these information's that you wanted in the manuscript). The value of the MIC is equal to the one of the MFC. In fact, the gelose of the MIC is seeded on a barren gelose made for that. After the incubation period, we still notice that there are not visible colonies on the agar gelose. Hence MIC = MFC. (We have included these information's that you wanted in the manuscript). Moreover, for every extract, there is a MFC value. These values are given in µg/mL to avoid a lot of numbers after the comma (example: extract T4-2, MFC = 24.37µg/mL which correspond after conversion to 0.002437 mg/mL). It's then easy to give this value in µg/mL. If not the next value would not more correspond to the multiple of 2 (according to the double dilution) when we start from the feeblest concentration to the highest concentration. For the positive control, the concentration is 0µg/mL because the witness tube of growth control doesn't contain vegetable extract. The growth for this tube is expressed in 100% of survival. We point it out in the manuscript. The content of the control growth tube is the positive control tube. Our objective is to evaluate and then optimize the antifungal activity of this plant; we have to reduce the colonies numbers as the activity is being optimized. We'd



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	rather say that from our method we'll better and better concentrated the active principle responsible of this activity. We owed you a lot of respect in this domain. We have read many of your publications which are not recent (since 1998). We do apologize. We wanted to show generally, but not to you the importance of cryptococcosis. Our method aim to allow us to optimize a biological plant activity. In the case of this plant, we had better results with the aqueous extract and other fungal germs such as <i>Candida albicans</i> (Yayé et al., 2011) and <i>Aspergillus fumigatus</i> (Yayé et al., 2012). Then is this precise case, the use of other organic solvents with different polarities could permit us to improve this activity which is already interesting? It's what we will do, and this has given us the expected results. In fact, we are not limited to some series of extract according to the solvent but the most active extract is optimized with other solvents. The methods that we have used (bioautography, quick microplate method) are different from ours (the different method of extraction, double dilution method). I thank you for your understanding