Author’s response to reviews

Title: Anti-aging potential of extracts from Sclerocarya birrea (A. Rich.) Hochst and its chemical profiling by UPLC-Q-TOF-MS

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Author’s response to reviews:

Barkat Ali Khan (Reviewer 1)

Comment: Abstract needs revision.

Action taken: Abstract has been revised to include statistical analysis (Page 2, lines 55-58)

Comment: Statistical should be incorporated to see the significant/insignificant differences between sample and standard

Action taken: Statistics have been incorporated to show the significant differences between the sample and the standard as suggested by the reviewer. Statistics were, however, not included for the first analysis in Table 1 as this was only the screening stage and the aim was to merely select the part of the plant showing the highest biological activity in the collagenase and elastase assay for further analysis.

Comment: The plant has been identified/authenticated by a botanist however botanist is not concerned to identification. it must be identified from a taxonomist.
Response: Prof A.E. van Wyk is a taxonomist and was for many years the curator of the Schweickerdt Herbarium (PRU) and MS Mothogoane is a taxonomist at South African National Biodiversity Institute (SANBI), Biosystematics Research and Biodiversity Collections Division, based at the National Herbarium (PRE). He has been working for the Institute for over 18 years and is responsible for over a 1500 plant species taxa. His employee number is 53386914.

Action taken: On page 6 line 147, the word botanist was changed to taxonomist and on page 6 line 158-159, the phrase curator and taxonomist were added.

Comment: The collected plant materials were oven-dried at 30-60 °C however heat drying is not supported as it may cause deterioration of biological activity. Why not use shade drying?? This point needs to be clarified scientifically.

Response: The CSIR undertook a high through-put plant collection campaign (many hundreds) and in order to avoid the storage of wet plants for long periods of time before drying due to space limitations, the plants were oven dried. Hence in order to avoid any possible decomposition through oven drying, we air dried the plants in the laboratory (shade) for further extraction and analysis in the subsequent collection.

Comment: It is missing in the Manuscript that which statistical tool has been used.

Action taken: The statistical tool used as been included in the manuscript on page 11 and 12 in the revised lines 292-297. The Student’s t-test function in Excel was used to compare the significance of the differences between the activity of the positive controls and the test samples.

Statistical analysis is added to Table 2, Figure 1, Figure 2 and Figure 4 and results and discussion (Page 13, lines 319 to 334; Page 14, lines 336 to 352; Page 17, lines 421 to 427; Page 18, lines 428 to 429)

Comment: Discussion is poor and not supporting the results

Action taken: Discussion has been revised with revisions highlighted in yellow on page 19 lines 458 to 466, line 469 to 471; page 20 lines 472 to 486, line 489; page 21 lines 518 to 519; page 23 lines 548 to 549, line 550, line 558 to 559).
Comment: Presentation is very weak.

Action taken: The authors have made efforts to strengthen the presentation by;

1. Improving the graphs in Figures 1, 2 and 4 by including the statistical information

2. The full spectrum of Marula stem ethanol extract was overlaid with an expanded chromatogram of the Marula stem ethanol extract showing the first 0 to 7 minutes region containing the major compounds in the extract so as to increase clarity and improve presentation of Figure 3.

3. The font sizes of all additional files and Figure 5 was increased and are now shown in black making them clearer, more visible thus improving their presentation.

Ching-Kuo Lee (Reviewer 2):

Comment 1. There are many errors in the manuscript

A. P8 line 213 20 mg/mL--→ 20 µg/mL

B. P9 line 234 20 ug/mL with 25 ug/mL order should be exchanged

C. P10 line 270 sodium formate 5 mM is right? (5uM??)

D. Table 1 and table 2 Control group should be placed in the first column

E. Table 2 10% MeOH and 10% DMSO repeats in Table 2 and should be combined

Actions taken

A. 20 mg/ml was corrected to 20µg/ml (Page 8, line 213)

B. The order of 20µg/ml and 25µg/ml was changed with 25µg/ml now appearing before 20µg/ml (Page 8, line 235 to 235).

C. 5mM sodium formate was used, so 5mM is right

D. We do not understand this suggestion. However, we have improved table 1 to be consistent with the table 2. The 10% MeOH values for elastase and collagenase are now in the same row.

E. The suggestion has been accepted and the table modified.
Comment 2. Effective active ingredient epigallocatechin gallate and epicatechin gallate cannot show a dose relationship at concentrations of 5 to 20 ug. Please explain why

Explanation: Our results showed a dose dependent response for elastase inhibition at 10 and 20 ug/ml for both epigallocatechin gallate and epicatechin gallate, but we cannot explain the lack of dose response at the 5 ug/ml at this point. Our results also showed a dose dependent response for collagenase at 5 and 10 ug/ml for both epigallocatechin gallate and epicatechin gallate, but we cannot explain the lack of dose response at the 20 ug/ml at this point.

Comment 3. Manuscript content is too long and repeated, should be substantially reduced and amended

Actions taken;
1. The orientation of Figure 3 was changed from landscape to portrait and its size was reduced appropriately.
2. The size of Figure 5 was reduced and the figure which was in landscape is now in portrait orientation with the peaks clearer in the new figure.
3. Table 3 was reduced from 4 pages to less than 1.5 pages after removal of unused space and lowering font sizes from Arial 11 to Arial 9.
4. The font size of Table 1, Table 2 and Table 4 have been reduced to from Arial 11 to Arial 9 to maintain uniformity with Table 3.
5. Repetitions have been removed as far as possible