Rebuttal

Review 1

1. Title be modify to: "Antiplasmodial potential of South African medicinal plants and phytochemical investigation of *Aloe marlothii*, *Turraea obtusifolia* and *Artemisia afra* for antiplasmodial principles".
   Response: Can't change the title

Chapter 3

2. Section 3.1, page 71, line 2: The names "Hemerocallidaceae", and "Asphodelaceae" are family names rather than for sub-families. The candidate is advised to provide the correct sub-family names for these two taxa.
   Response: corrected (Page 71)
3. Page 90, Figure 3.6: i) correct the structure of prechrysophanol, add methyl group at C-3. ii) How was it possible to define the configuration using UPLC-QTOF-MS? Iii) is it possible to distinguish prechrysophanol from aloesaponol II using mass spectrometry? If so how?
   Response: i) structure corrected (Figure 3.6, Page 90). ii) The configuration was not determined. Compounds were tentatively identified based on the MS/MS fragmentation pattern in comparison with published data and also based on previous report of identification of those compounds in the species or genus. iii) it is not possible to distinguish prechrysophanol from aloesaponol II using mass spectrometry, this is why I suggested that the peak should be isolated and structure determined using NMR.
4. Page 87, paragraph 1, line 1: aloesaponol I (45), is a pre-anthraquinone, but not an anthraquinone.
   Response: Corrected as recommended (Page 88, line 1)
5. Page 87, paragraph 1, Line 5: change "monoxides" to "carbon monoxides".
   Response: Corrected as recommended (Page 87-88)
6. Page 87, paragraph 2, Line 8: How can an 'ethyl molecule' be lost from prechrysophanol in its MS fragmentation? The candidate is advised to check the fragmentation in the MS more carefully
   Response: Corrected (Page 88)
7. Page 91, Section 3.3.3.1, line 6: change 'methoxy group attached to the carbonyl group' to 'methyl ester'
   Response: Corrected as recommended (Page 91)
8. Page 91, Section 3.3.3.1, lines 11 and 19: change 'oximethine' to 'oxymethine
   Response: Corrected as recommended (Page 91)
9. Page 92, Figure 3.7: How was the configuration at C-3 of compound 45 determined without ECD or CD data generated and compared with literature?
   Response: The relative configuration was deduced from coupling constants which were compared with the literature (Page 92).
10. Page 92, Table 3.2: Change 'MeOD-d4' to 'CD3OD' and 'DMSO' to 'DMSO-d6'
    Response: Corrected as recommended (Table 3.2, Page 92)
11. Page 93, Section 3.3.3.2, line 6: the spin system designation 'ABC' for the signals at H 7.31 (1H, dd, J = 1.07, 8.31 Hz, H-5), 7.62 (1H, t, J = 8.10 Hz, H-6), and 7.77 (1H, dd, J = 1.07, 7.50 Hz, H-7) appears to be wrong, the pattern appears to be 'AMX'; the candidate is advised to check. The same comment is applicable in the discussion on the structure of chrysophanol. Section 3.3.3.3.,

line 2: the calculated value given for C15H11O4, as "255.2460" is wrong; the correct calculated value is '255.0657' which is in agreement with the experimental exact mass of "255.0640" obtained for this compound (48) as shown in line 2 of this section

Response: i) The spin system designation 'ABC' in the thesis is correct (Some references: 1. Ahmad R, Shaari K, Lajis NH, Hamzah AS, Ismail NH, Kitajima M: Anthraquinones from Hedyotis capitellata. Phytochemistry 2005, 66(10):1141-1147; 2. Hou Y, Cao S, Brodie PJ, Callmander MW, Ratovoson F, Rakotobe EA, Rasamison VE, Ratsimbason M, Alumasa JN, Roepe PD et al: Antiproliferative and antimalarial anthraquinones of Scutia myrtina from the Madagascar forest. Bioorganic & Medicinal Chemistry 2009, 17(7):2871-2876).

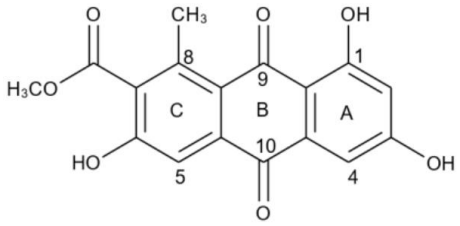
ii) The calculated value of $C_{15}H_{11}O_4$ has been corrected (Page 94).

12. Page 95, Section 3.3.3.3, line 10: change 'hydroxyl' to 'hydroxy'. This comment is applicable to seven other places in the thesis.
    Response: Corrected as recommended
13. Page 96, Table 3.4: the 13C NMR chemical shift values are given to two decimal places for some and to one decimal place for other carbon atoms. The candidate should be consistent throughout the thesis on the use of the number of decimal places.
    Response: Corrected to one decimal for literature values everywhere
14. Page 97, Section 3.3.3.4, line 8: give the unit of coupling constant as 'Hz'. Also give the J values to one decimal place and consistently throughout the thesis
    Response: Corrected to one decimal throughout the thesis
15. Section 3.3.3.4, line 17: The absolute configuration of aloesaponol IV cannot be deduced from 1H NMR spectrum. The candidate should have compared the ECD and/or OR data with literature after confirming the relative configuration based on 1H NMR spectroscopy
    Response: The relative configuration of aloesaponol IV was deduced by comparing the coupling constant with the ones published in literature (Page 96).
16. Page 98, Table 3.5: is the 13C NMR spectral data for aloesaponol IV reported here for the first time? If that is the case, this should be indicated. On the other hand, if it has been reported, include the 13C NMR spectral data from literature in Table 3.5 for comparison
    Response: The ^{13}C NMR spectral data for aloesaponol IV are reported for the first time. This is indicated in the thesis (Table 3.5, Page 97)
17. Page 99, Section 3.3.3.5: Change 'β-sorigenin-1-O-methylether' to 'β-sorigenin-1-O-methyl ether' i.e. the word 'ether' should be written as a separate word.
    Response: Corrected as recommended in the whole thesis
18. Page 100, Figure 3.11. The ^{4}J HMBC correlation shown between H-4 and C-1 is unlikely; such ^{4}J correlation could only be between H-4 and C-8 where 'W-coupling' is possible. May be, the assignment of C-1 and C-8 should be interchanged. The candidate is advised to check carefully the HMBC spectrum for this compound.
    Response: There is HMBC correlation between H-4 and C-1. C-1 and C-8 cannot be interchanged. A methyl ester group is attached to C-8. Additionally, There are HMBC correlations of both OH, H-6 and H-7 with C-8 (Figure 3.11, Page 98).
19. Page 101, Table 3.6: the normal value for methoxy signal in 13C NMR occurs at around 55 ppm. In this compound the methoxy signal occurs at 65.05 ppm. Even the 1H NMR signal for this methoxy group is abnormally high (4.41 ppm). Can the candidate explain these 'abnormal' values?

Response: The unusual chemical shift is observed for out-of-plane methoxy groups (Reference: Toušek J, Straka M, Sklenář V, Marek R: Origin of the Conformational Modulation of the 13C NMR Chemical Shift of Methoxy Groups in Aromatic Natural Compounds. The Journal of Physical Chemistry A 2013, 117(3):661-669)

20. Page 101, Section 3.3.3.6 : In my opinion, compound 53 is not emodin, rather it is helminthosporin (structures below) for the following reasons: i) There is no report on the occurrence of C-6 oxygenated anthraquinones from Aloe species. The claim by the candidate that compound 53 is emodin was not supported by the data presented; ii) emodin having a free phenolic group will not be readily soluble in CH_2Cl_2. On the other hand in helminthosporin, all the three hydroxy groups being involved in intramolecular H-bonding, the compound is fairly non-polar and hence soluble in CH_2Cl_2; iii) the colour described for compound 53 as "deep red" and the presence of three highly deshilded OH signals in the ^{1}H NMR spectrum is consistent with this compound being helminthosporin rather than emodin, thus in ^{1}H NMR spectrum, H-6 and H-7 appeared as AB quartet at ca. 7.31, while H-5 appears at 7.71 ppm which also shows long range coupling with Me-3. It should be noted that, wrong structure proposal has an implication on the discussion on the biological activity of compound 53 where the candidate has tried to explain the difference in the level of activity of this compound in comparison (page 105, last paragraph, line 5 from the bottom) with what has been reported for emodin. Now that compound 53 is helminthosporin but not emodin, this discussion has to be revised.

Response: The compound is indeed emodin (Page 100). i) There are reports of C-6 oxygenated anthraquinones isolated from Aloe species, e.g. Laccaicacid D methyl ester (structure below) (https://doi.org/10.1016/0031-9422(92)83149-S, https://doi.org/10.1016/j.phytol.2012.04.014, https://doi.org/10.3390/molecules19033264) .

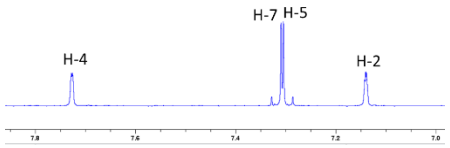


Laccaicacid D methyl ester

Furthermore, emodin had previously been isolated from some Aloe species (References: https://doi.org/10.1080/14786410802242851, https://doi.org/10.1080/14786410802242851)

ii) The are many reports of emodin NMR data recorded in chloroform (https://doi.org/10.1007/s10600-021-03366-2, https://doi.org/10.1007/s10600-019-02651-5, https://doi.org/10.1080/14786419.2019.1684281).

iii) The two compounds are described in the literature as "orange". Therefore, the color is the same for both (https://doi.org/10.1021/acsomega.9b03693). The proton at 7.73 ppm is H-4 and not H-5



^{1}H NMR aromatic region for compound 53

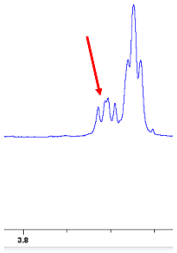
21. Page 106, Table 3.9: include the IC_{50} value of the crude extract and comment how this compares with the activity of the pure compounds. For aloesaponarin I, give the IC_{50} values in µM to the same number of significant digits
    Response: Extract was screened in a dual point assay at 10 and 20 µg/mL. The IC_{50} was not determined. Therefore, there is no way to include the value in the Table. The IC_{50} value in µM for aloesaponarin I has been corrected (number of significant digit). Page 103, Table 3.9.
22. Page 108, Section 3.3.5: alosaponarin I is cytotoxic, which indicates that the observed antiplasmodial activity is due to general cytotoxicity. If that is the case, was the molecular docking necessary considering that?
    Response: I didn't test the toxicity of alosaponarin I, neither did I see it mentioned in the literature. Furthermore, It did not show any activity against the gametocytes, which does not support the hypothesis that the antiplasmodial activity is due to general cytotoxicity.

Chapter 4

1. Page 127, Section 4.2.1: The solvent used for extraction of *T. obtusifolia* leaves is acetone, while the solvent used to extract *Aloe marlothii* was CH2Cl2/MeOH (1:1), why the difference?
   Response: *T. obtusifolia* was included based of previous work done in the lab, where it showed good activity and we needed to use a hyphenated approach to isolate the active ingredients. The acetone extract was used for the previous test and we decided to stick to that.
2. Page 128, line 3: The candidate is advised not to begin a sentence with a numeral; this sentence should be reworded.
   Response: Corrected as recommended (page 127)
3. Page 136: give the IC50 values to the same number of decimal places consistently.
   Response: Corrected to one decimal for consistency (Page 135)
4. Page 139, Section 4.3.5.1: avoid the use of the word "novel", instead you may use the word 'new' or 'previously undescribed'
   Response: Changed from "novel" to "new" (Page 138)
5. Page 139, Section 4.3.5.1, line 3: change "$C_{21}H_{34}O_4$" to "$C_{21}H_{35}O_4$"
   Response: Did not changed as recommended because the molecular formula is $C_{21}H_{34}O_4$ (Page 138)
6. Page 140, paragraph 2, lines 2 and 3: It was indicated that "In the HMBC spectrum, the methyl at δ_H 1.11 (19-CH_3) correlated with two oxygenated carbons appearing at δ_C 67.28 (C-1) and 74.70 (C-5), …" however, in the structure shown, there is no hydroxy group located at C-1. The candidate is advised to look at the structure proposed carefully and make the correct conclusion on the placement of the hydroxy group, i.e. should it be at C-1 or C-2? In the same token, the candidate is advised to carefully study the NOESY and HMBC spectra so that the placement and configuration of the hydroxy groups are well established.
   Response: Corrected to C-2, it was a mistake as C-1 was already assigned to δ_C 35.33 (Page 139)

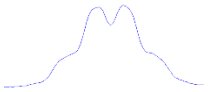
7. Page 142, Table 4.3: i) The description of multiplicity of some signals should be looked at more carefully. For example, the multiplicity for H-2 should be 'ddd', as it has three coupling partners, thus the 'dd' indicated in the table is wrong; ii) the values of the coupling constants given for H-2 as '3.18, 4.80 Hz' does not correspond to this proton which is axially oriented as shown in Figure 4.11 (b); iii) the multiplicity in the 1H NMR signal for H-3 at ?H 4.02 is depicted as "q" with a coupling constant of 3.18 Hz). This is wrong and it couuld only be 'ddd' having three coupling partners with three coupling constants; iv) change "Cosy" to "COSY" ; v) the multiplicities of several of the 1H NMR signals are expressed as "m", the candidate is encouraged to analyze the 1H NMR spectra and provide the appropriate descriptions along with the coupling constants, which will allow the candidate determine the relative configurations at various stereocentres; the signal for H-6 at ?H 3.67 is described as "t, 3.24 (Hz)" This proton being axially oriented the multiplicity cannot be triplet, furthermore for axially oriented proton the coupling constant, i.e. J6ax,7ax should be large, which again put in question the correctness of the structure proposed and the assignment of the NMR data; vii) the candidate should include the NMR data of 2β,3β,5β-trihydroxy-pregn-20-en-6-one in Table 4.3 so that direct comparison can be made on these two compounds.

   Response: i) the multiplicity of H-2 is not well defined (see picture below). Changed in the thesis from "dd" to "m" (Page 138)



   ii) Corrected

   iii) Changed the multiplicity from "q" to "m" because no clear pattern as shown on the picture below (Page 138)



   iv) Corrected Cosy to COSY (Page 141)

   v) 1H NMR spectra of pregnane steroids show a lot of overlaps, that's why most of them are described as "multiplet" (References: 1. Yuan C-M, Tang G-H, Wang X-Y, Zhang Y, Cao M-M, Li X-H, Li Y, Li S-L, Di Y-T, He H-P et al: New steroids and sesquiterpene from *Turraea pubescens*. Fitoterapia 2013, 90:119-125. 2. Wang X-N, Fan C-Q, Yue J-M: New pregnane steroids from *Turraea pubescens*. Steroids 2006, 71(8):720-724)

   vi) Corrected as recommended: the NMR data of 2β,3β,5β-trihydroxy-pregn-20-en-6-one has been added in Table 4.3 (Page 141).

8. Page 141, Figure 4.11: the distance between H-9 and H-14 appears to be far for NOE to be observed. The candidate should show the conformation to prove that the observed NOE is between these two protons. Otherwise, this may imply the assignment of 1H NMR signals could be wrong

Response: I didn't show any NOE between H-9 and H-14 in Figure 4.11 (Page 140)

9. Page 143, section 4.3.5.2: Compound 79 was isolated a "yellow powder" which indicated that the compound was not isolated in a pure form (a pure steroid should be colourless as there is no conjugation). The NMR spectra also indicate that the sample may not be pure. Furthermore, the description of the multiplicities (Table 4.4) for some of the protons are not consistent with the structure proposed. For example, H-6 at H 3.97 (brs, 1H) and H 3.91 (brs, 1H), both protons shown to be axially oriented are expected to show large coupling constant, but not as "broad singlet" as indicated in Table 4.4 for these protons. This shows the assigned configurations; by extension the structure proposed, may not have been supported by NMR evidence. It should be noted that this compound is claimed to be new, and hence the evidence should be water tight.
Response: The compound may have some impurity that gives the color. I attached the 1H NMR and 13C NMR spectra below. The multiplicities were not well resolved.

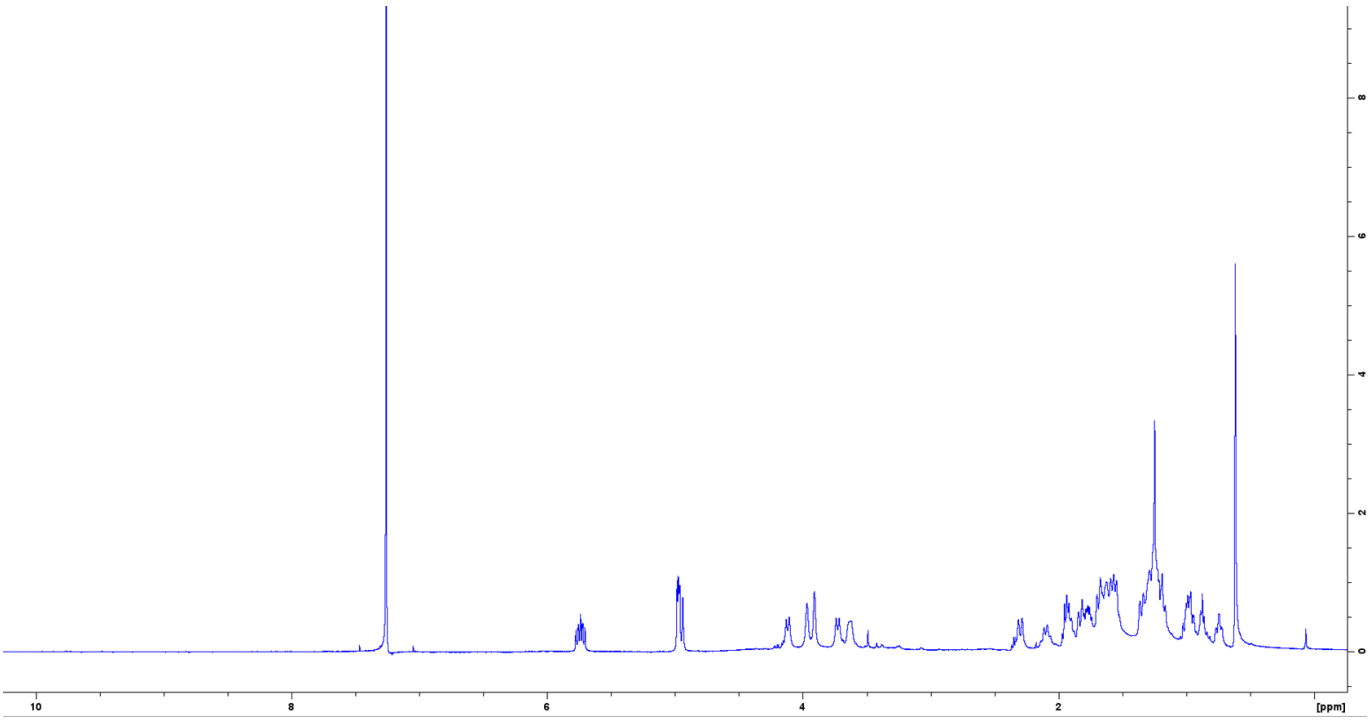


Fig: ^{1}H NMR spectrum of compound 79

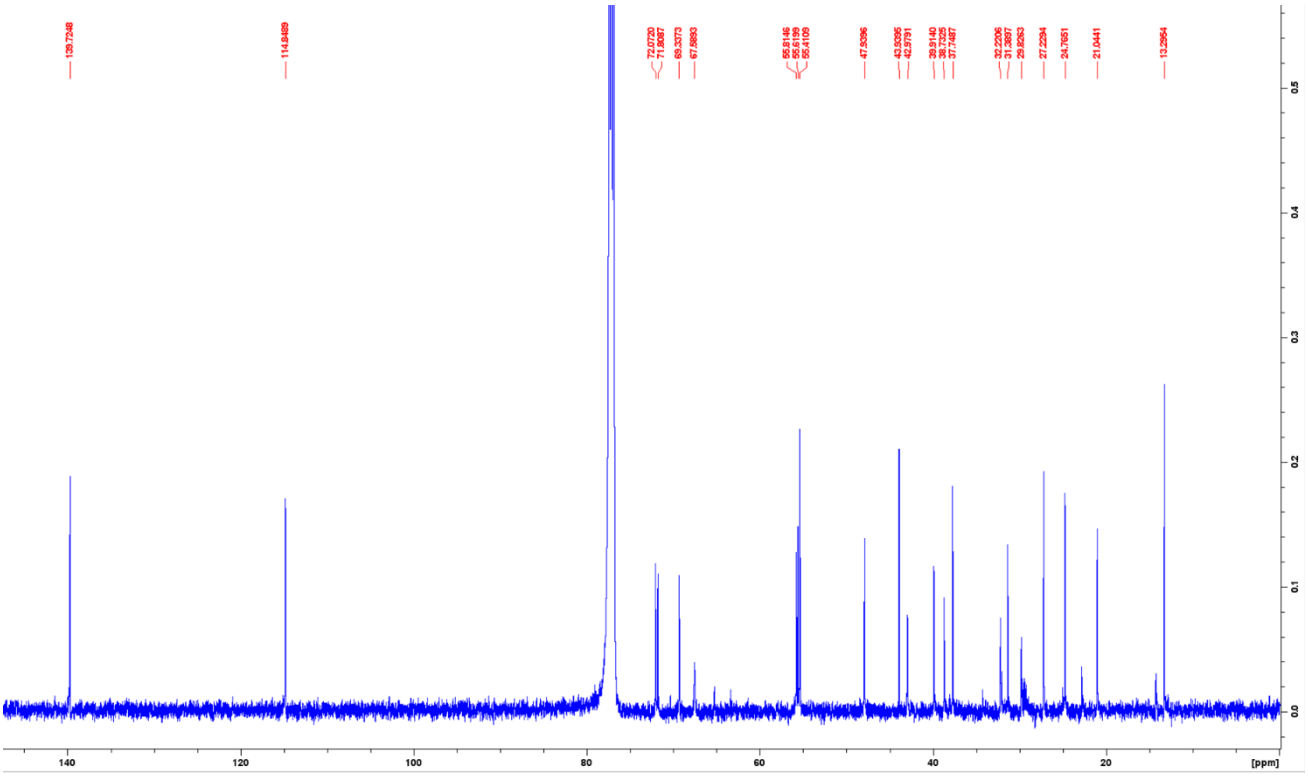
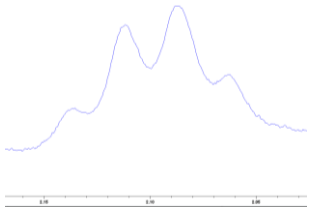


Fig: ^{13}C NMR spectrum of compound 79

10. Page 144, line 2: in describing the ^{1}H NMR data and multiplicity of H-4a in compound 79, the candidate indicated "The methylene protons at δ_H 2.10 (1H, q, J = 12.57 Hz, H-4a) …" the description 'quartet' imply that the coupling partner is a 'methyl' group which is not the case here. The candidate should carefully go through the NMR data and use the correct descriptions and coupling constants for this compound.
    Response: Multiplicity changed to dd, J = 12.57, 12.02 Hz (See picture below), Page 143.

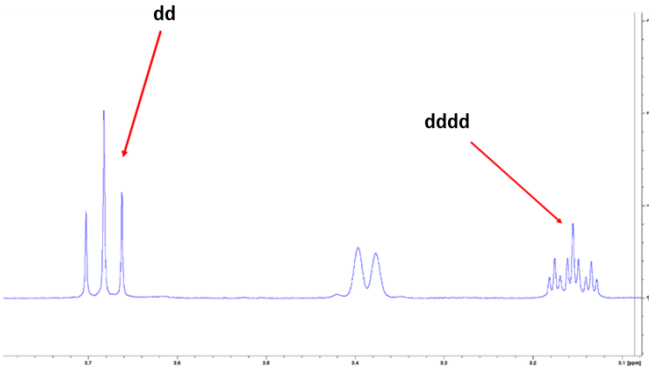


11. In Supplementary data 47 (^{13}C NMR) and 48 (DEPT) for compound 79, the signal for C-19 is weak to be considered as a signal for the major compound. That the sample is impure is also evident from the NMR spectra. Considering the above shortcomings, the candidate has not proved the identity of compound 79 unequivocally. I suggest this compound be purified and re-run in another solvent, e.g. acetone-d6 and the proposed structure should be confirmed or revised before publication.
    Response: Noted.

12. The comparison of compounds 81-84 with literature fits well. However, in the supplementary data the ^{1}H NMR spectrum of rubralin B (81) is too complex, the signals are not integrated and, in my opinion, the sample does not look pure. I am not sure how the candidate was able to propose the structures with configuration defined at several stereocenters without generating ECD and OR data. Has the candidate generated ECD and OR, and compared these with literature? The candidate should also comment on the biogenesis of these compounds.
    Response: Rubralin is a large molecule with 58 protons hence it appeared complex (Table 4.7, Page 157, 1H NMR Supplementary data 68, Page 266).
13. The antiplasmodial activity of the crude extract, fractions and pure compounds obtained from T. obtusifolia leaves have been investigated. The most active compound being rubralin B (82), with an IC50 value 4.47 µg/mL. This compound being complex, the candidate should comment on its potential as a lead compound in the development of antimalarial drug(s).
    Response: Limonoids are very complex, which makes it challenging for synthesis if considered as lead. SAR studies would be helpful to identified relevant parts of the molecule for synthesis of less complex derivatives.

Chapter 5

1. Page 194, Section 5.3.5.1, line 2: the candidate has proposed a molecular formula as $C_{17}H_{20}O_4$ for zuurbergenin (90), based on ^{1}H and ^{13}C NMR spectral data alone. In my opinion this is wrong. NMR data could only be used to support high resolution MS in determining molecular formula.
    Response: The molecular formula was deduced from both MS and NMR. I just indicated that in the UPLC-MS, the *m/z* observed corresponded to a fragment and not the molecular ion.
2. Page 196, Table 5.3: the candidate should check the multiplicity and coupling constants for some of the protons. For example, why would H-6 have a multiplicity of "tt" with J value of 3.02, 10.32 Hz; for the same proton literature data description of multiplicity is "dddd" with J value of " J7,8 = 10 Hz". In my opinion, both descriptions are wrong. The candidate should check the spectrum as well as the literature description for all the proton in this compound carefully and make corrections. Has the ECD and/or OR of zuurbergenin and costunolide isolated here compared with literature so that the absolute configuration is also proposed.
    Response: Change to dd and dddd (Table 5.3, Page 196). ECD and/or OR were not measured but I didn't have access to instruments.



3. Page 200, Table 5.5: How can the multiplicity for H-7 at δ_H 2,71 be described as "br" having three coupling partners? The candidate should check the spectra carefully and provide the appropriate description of multiplicity and coupling constants for this proton as well as others.

Response: The peak was not well resolved. This may be due to the solvent or the NMR instrument parameters (Table 5.5, Page 200).

4. Page 201, line 1: the difference between the experimental and calculated value should not exceed 10 ppm for determining molecular formula from MS. How sure is the candidate on the correctness of the molecular formula? The candidate is advised to recalculate the theoretical mass
   Response: The molecular formula is further supported by ^{1}H and ^{13}C NMR data.
5. Page 201, line 1: the calculated value given for the molecular formula $C_{19}H_{31}O_3$ here appears to be wrong and it should read as '307.2273' Again, the candidate is advised to recalculate the theoretical mass for this compound as well.
   Response: Corrected as recommended (Page 203).
6. Page 203, Section 5.3.5.5: in the structure for 12-oxo-phytodienoic acid methyl ester (94), the candidate should comment on how the configuration (relative or absolute) was determined. Has the ECD and/or OR of the isolated compound compared with literature data?
   Response: The NMR data were compared to the published data for 12-oxo-phytodienoic acid methyl ester (Page 203). The ECD and/or OR were not measured.
7. Page 206, Figure 5.11: keeping in mind that ^{4}J correlations are not common in HMBC spectra, can the candidate explain the ^{4}J HMBC correlation shown here between H-6 and C-1.
   Response: The "W" shape of that part of the molecule.
8. Page 206, Figure 5.12: acacetin (85) is a well-known compound whose structure can be elucidated by NMR and MS. What was the reason for generating Crystal structure?
   Response: The compound was isolated as crystal. It was then recrystallized to obtain of purer compound, hence the crystal structure was determined and the NMR data were analyzed to confirm the structure and absolute configuration (Page 206).
9. Page 213, Table 5.11: in arteanoflavone (96), the 6-OCH_3 group appear at δ_C 61.04 which is at low field compared to most methoxy resonances (55-57 ppm). Can the candidate explain why the value appeared at low field?
   Response: As mentioned above, the unusual chemical shift (δ_C 61.04) is observed for out-of-plane methoxy groups (Reference: Toušek J, Straka M, Sklenář V, Marek R: Origin of the Conformational Modulation of the ^{13}C NMR Chemical Shift of Methoxy Groups in Aromatic Natural Compounds. The Journal of Physical Chemistry A 2013, 117(3):661-669).
10. Page 218, section 5.36 and Table 5.14: with regards to the results of the antiplasmodial activity of compounds isolated from *A. afra*, some of the compounds which showed good activity have already been reported to show antiplasmodial activities. Especially, the antiplasmodial activity of flavones are well documented. In this regards, the re-isolation and re-establishment of common flavones as antiplasmodial agents, from this well-known medicinal plant did not support the alleged superiority of the hyphenated method followed in this study over the classical methods
    Response: The time to isolate the compounds was significantly reduced.

Chapter 6:

In this chapter the candidate has made "General conclusion". However, in my opinion, a conclusion should entirely be based on the results obtained in the present research. In the first paragraph of this chapter, the statements are too general and are known facts, even before the present research was conducted. These are statements used in introductory chapters as justification for the search of new antimalarial lead compounds from plants. Over all, this chapter is written as summary rather than conclusion. If the title

"General conclusion" is to be retained for this chapter, then only the most important finds of the present study should be written in a form of Conclusion, or the title of this chapter be changed to 'summary' depending on the format recommended by the Faculty.

Response: Corrected

References

The references are listed at the end of each chapter and appear to be consistent. I hope the format followed is that of the Faculty. The candidate is advised to double check if all the references mentioned are listed and vice versa.

Response: Done

Supplementary data

The Mass and NMR spectra are attached in as Supplementary data. However, I suggest the title for this section should be titled as 'Appendices' which is commonly used in thesis writing.

Response: Title changed from "supplementary data" to "Appendix" (Page 233).

Review 2

1. The summary, it's too long and contains too many details
   Response:
   - It is recommended to add the family name after the active plant.
     Response: Added as recommended (Page V)
   - Page V. The inclusion of *Turraea obtusifolia* and *Artemisia afra* in the working plan has no apparent justification, is it traditional or previous scientific reports?
     Response: These were previously screened and showed good activity, hence they were included for further investigation.
   - Page V. β-sorigenin-1-O-methylether, O is italic; at 10 and 20 µg/ml, Liter is L (capital)
     Response: Corrected (Page V)
   - Page .4. there are different statements for the aim of the study. For example, on page 4, "The aims of this study were to contribute to the building of the South African national natural product library through the investigation of a selected subset of plant materials from this repository and to evaluate the antiplasmodial activity of samples generated for the library and to isolate and characterize biologically active compounds using modern hyphenated analytical techniques from selected species. // Page 8. This study focuses on medicinal plant species from South Africa, for which the record of traditional use in the treatment of malaria and associated symptoms (fever mainly) exists. The aim of the study needs to be the same, especially to know if the main entry of the project is traditional- or literature-based, or random screening based.
     Response: The statement on page 8 does not enunciate the aim of the study. The section 1.4 was about Traditional uses of plants in the fight against malaria. Many plants from various area were listed. So, we it was necessary to indicate that in our study, only plan species from South Africa were included.
2. Abbreviations: NOESY Nuclear Overhauser effect spectrometry, please correct.

Response: Corrected (Page XX)

3. Page 2. Figure 1.1. compounds 3, 4, and 5, please add the stereochemistry
   Response: The stereochemistry is shown in Figure 1.1 for compounds 3, 4 and 5 (Page 2).
4. In figure 1.4, please add the explanation of "*" in compounds 7 and 8.
   Response: Added (Figure 1.4, Page 10)
5. Page 23. South Africa is ranked as the third most biodiverse country in the world after Indonesia and Brazil [1]. I think S.A. is ranked 15 according to the new list; please check the recent literature carefully again.
   Response: The rankings are different. In one case SA is ranked 13, in another, it's 19. Therefore, I have removed the ranking in the thesis (Page 23).
6. Page 28. The selection criteria may need further improvement in the future and mix between the bullets, especially those related to traditional uses (1, 2, and 6). Also, it's recommended to add the conservation status to the criteria in addition to the link between south African and the plants (if the traditional use of the plant is reported by another nation rather than South Africa may weak the selection due to environmental factors).
   Response: Noted
7. Page 28. "In cases where the selected species was not found in the repository, it was subsequently replaced by another species from the same genus." This is not logical, there is no guarantee to have the same activity (please correct the highlighted words.
   Response: Corrected
8. Page 28. The plant and extract repository was created during the period between late 1998 and 2005 at the Council for Scientific and Industrial Research (CSIR). This period is long, please state the way of storing the plant materials.
   Response: Plant materials were stored in powder form at room temperature.
9. Page 29. Dry extracts (150 – 250 mg) were dissolved in 4 mL of a mixture of MeOH, ethyl acetate (EtOAc), and methyl tert-butyl ether (MeOH/EtOAc/MTBE) (6:3:1). The candidate needs to explain why this ratio was selected.
   Response: It's indicated in the thesis that the fractionation method implemented was based on the one published by US National Cancer Institute (NCI) (Section 2.2.2, Page 29).
10. Table 2.1. the candidate must verify the names and authors correctly, and the original (accepted) names (not synonyms) should be selected. Please check according to https://wfoplantlist.org/plant-list
    Responded: Done
11. Table 2.1. there are many plants missing the supporting references (e.g. C. steenkampianus/ E. natalensis).
    Response: Because there were no references for these plants (Table 2.1, Pages 41, 44, 45).
12. Both tables 2.1. and 2.2 are missing *A. afra* and *T. obtusifolia*
    Response: Because the extraction was done differently and the antiplasmodial activities of plants in the tables were determined at dual points (10 and 20 µg/mL), while for *A. afra* and *T. obtusifolia*, the IC_{50} were determined.
13. Figure 2.4. the structure of compound 32 is wrong.
    Response: Corrected (Figure 2.4, Page 56).
14. Page 61. Additionally, Turraea obtusifolia and Artemisia afra, which were also tested for their antiplasmodial activity, were further explored to identify their active compounds. The

antiplasmodial activity of their extracts and subsequent fractions were not discussed in this current chapter because these were extracted with acetone and not DCM:MeOH and MeOH as the rest of the plant materials. The candidate needs to explain why the two plants were inserted, at the same time, other plants have been tested and showed more interesting activity than these two plants.

Response: These were added based on work done previously in the lab. They showed good activities worth investigating further.

15. Page 75. Figure 3.2. the candidate is confused with the stereochemistry of the glucose unit; please revise and correct.

Response: Corrected Fig. 3.3 (Page 76).

16. Page 83. The injection volumes were 200-400 µL. it is recommended to work with weights, not volume.

Response: Noted

17. Page 90. Figure 3.6. the structure of Precysophanol is wrong.

Response: Corrected (Figure 3.6, Page 90).

18. In general, please apply for ALL NMR TABLES, the C-13 chemical shifts are reported using one digit, while, H uses two digits and J values in one digit (e.g. δ_C 120.1; δ_H 7.22 ppm and J=7.6 Hz), what is reported in the thesis is not common, and is not acceptable by the majority of the natural products related journals.

Response: Applied

19. Page 91. There is no Ha and Hb in the cyclic system, it should be alpha (α) and beta (β). This applies to this compound and others in the thesis (to be corrected).

Response: Corrected where applicable

20. Page 92. The relative and absolute configuration can be easily determined since the compound contains only one asymmetric center. The candidate needs to discuss this and propose the right stereochemistry

Response: The stereochemistry was deduced from comparing the coupling constant with the published compound.

21. Page 91. δH 2.74 (1H, dd, J = 17.5, 7.2 Hz, H-2a) and 2.97 (1H, d, J = 7.6 Hz, H-2b). H2b missing germinal coupling.

Response: The peak was not well resolved and there was overlaps with H-4a (Page 91)

22. Page 91. δH 2.97 (1H, dd, J = 13.3, 3.26 Hz, H-3a) and 3.20 (1H, dd, J = 15.5, 3.26 Hz, H-3b) for the second. It should read 4a and 4b, not 3a and 3b.

Response: Corrected (Page 91)

23. Page 93. Table 3.2. there is a problem in the assignment of carbons C-7, 8, 8a, 9a, and 10a. an affirmative decision should be made by the candidate to decide which one is correct (the experimental data or the reported ones).

Response: There was no problem. The chemical shifts are slightly different from reported ones

24. Page 94. Table 3.3. The assignments of C-2, 9a, and 10a are different from the reported values, discussion, and decisions that the candidate should make.

Response: Maybe influenced by the instrument parameters.

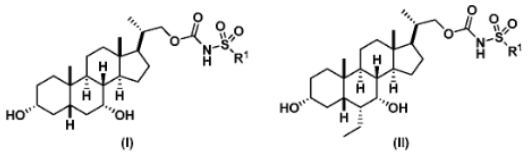
25. Page 97. meta COSY correlations. There is nothing called meta COSY; please remove meta, and use COSY correlations or cross-peaks. For H-5 and H-7, there is no coupling, according to table 3.5

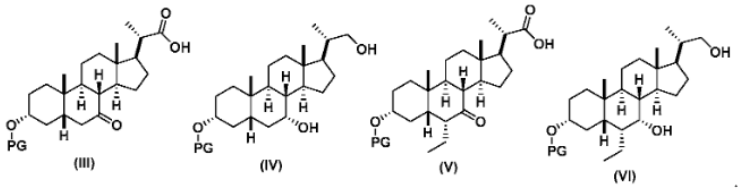
Response: Corrected.

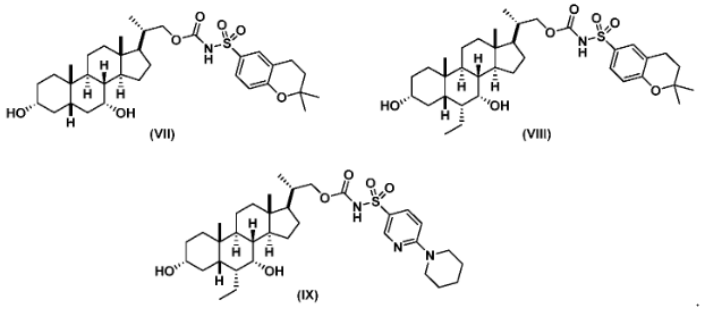
26. Page 97. The absolute configuration was deduced from ^{1}H NMR spectrum by analysing the coupling constants between H-2 and H-3 (H-2, Jaa = 9.6, Jae = 4.41) and H-4 and H-3 (H-4, Jaa = 5.94, Jae = 3.38). These values were equivalent to that reported for aloesaponol IV, for which the configuration was established as 2-axial and 4-quasi-axial [65]. Normal NMR experiments can't determine the absolute configuration. Please revise and correct the sentence.
    Response: Corrected to "relative" configuration (Page 96)
27. Page 99. 7.38 (1H, d, J = 8.2 Hz, H-7) and 6.93 (1H, d, J = 7.61 Hz, H-5), please correct according to table (3.6).
    Response: Corrected (Page 98)
28. Page 100. isoleutherol-4-O-glucoside. O is italic
    Response: Corrected (Page 99)
29. Page 101. orange-red solid. Solid is not the right description; please indicate either powder or crystalline. Please check other compounds
    Response: Corrected.
30. Page 101. meta COSY interactions between H-2 and H4. Please use the right expression. Also, there is no coupling, according to table 3.7.
    Response: Corrected (Page 100).
31. Page 103. both the H-2 and H-4 and 3-CH3 with C-2 (δ_C 149.54)., this should be C-3.
    Response: Corrected (Page 101)
32. Page 103. correlations of 1-OH to C-1 (δC 163.08) and C-2, H-6 to C-5 (δC 161.26) and 8-OH to C-7 (δC 157.74). in the case of OH-5, please use the HMBC of OH-5 rather than the correlation of the vicinal proton(s).
    Response: OH-5 did not show HMBC correlations (Page 101)
33. Page 105. β-Sorigenin-1-O-methylether (52). O italic.
    Response: Corrected (Page 105)
34. Page 110. The conclusion ignored the SAR; even if not in-depth, this needs to be highlighted.
    Response: The conclusion included the potential targets of compounds (Page 109)
35. Page 124. Beta-sitosterol, please correct the stereochemistry of the side chain.
    Response: Corrected (Figure 4.6, Page 123).
36. Pages 139-143 (Please apply to other compounds in the thesis). The structural elucidation of 78 looks good. However, the candidate needs to indicate the configuration of H-21a and 21b (trans/cis). Please indicate α or β configuration for each of every proton in the cyclic systems. The relative configuration of the compound needs more work before publication since the given criteria (using NOESY) are not sufficient and can be applied to other configurations as well. I propose to run an X-ray for one of the isolated compounds to build up the story correctly. The 3D energy-minimized structure(s) can also assist in the clarification of the stereochemistry. Please re-check the coupling constants for all protons (for example H-17, H-20, H-21a).
    Response: H-21a and H-21b are attached to the same carbon. I don't understand what's meant by trans/cis in this case (Page 138). The α and β have been indicated.
37. Page 139. Further, in the 1H NMR spectrum, three oxygenated methine protons were resonating at δH 4.02 (1H, q, J = 3.18 Hz, H-2), 3.70 (1H, dd, J = 3.18, 4.80 Hz, H-1), and 3.67 (1H, t, J = 3.24 Hz, H-6). Please recheck and correct.
    Response: Corrected (Page 138).

38. Page 140. In the HMBC spectrum, the methyl at δH 1.11 (19-CH3) correlated with two oxygenated carbons appearing at δC 67.28 (C-1) and 74.70 (C-5). one quaternary carbon (δC 43.05), one methine (δC 30.43), and one methylene (δC 35.19), which were assigned at positions 1, 5, 10, 8 and 11, respectively. This isn't very clear, and the HMBC correlations are confusing. First, if C-1 is oxygenated (not C-2). This contradicts the final structure, and the structural elucidation needs to be re-structured again. Please re-check again "carefully" the HMBC. Second, the HMBC correlations in the aliphatic system don't exceed the three bonds and are very rare to extend to the fourth bond (only in a few cases of conjugated systems), so the relations with C-11 (35.19) and C-8 (30.34) are not right
    Response: There was a mistake, C-2 is the one oxygenated (this has been corrected). C-11 and C-8 are three bonds away (Page 139).
39. Page 140. proton at δH 4.02 (H-2) with C-5, C-8, and C-10. proton at δH 1.69 (1H, m, H-4a) with C-1 and C-10, proton at δC 1.80 (1H, m, H-3b) with C-1 and C-5, and proton at δH 4.02 (H-2) with C-5 helped to assign the rings A and B. Please recheck
    Response: Corrected (Page 139).
40. The relative stereochemistry of compound 78 was established by NOESY spectrum (Figure 4.10), 1H, and 13C NMR. The NMR data for 18-CH_3 (δ_H 0.60, δC 13.26) were consistent with a it being β-orientated and the rings C/D trans-fused, as reported for pregnane steroids [45, 46]. This is not enough, as mentioned early, please correct the sentence
    Response: I'm talking about "relative stereochemistry". So, the sentence is correct.
41. Moreover, from the biosynthesis of pregnanes, the 18-CH3 and 19-CH3 are always β-oriented [47,48]. This is not correct; the background on the plant through the introduction mentioned many other configurations.
    Response: The introduction covered limonoids and not pregnanes steroids.
42. Page 144. The relative configuration, as mentioned above, needs more support. Also the assignments of all protons should be done correctly as α and β.
    Response: Noted
43. page 146. twelve sp3 methines (one aldehyde at δC 161.11, four oxygenated at δC 70.70, 71.36, 74.26. please correct (remove SP^3).
    Response: Corrected
44. Compound 68. Please correct the numbering of the furan ring; as mentioned earlier, the candidate must write the correct configuration of the protons.
    Response: Corrected (Figure 4.14, Page 148).
45. Page 147. δ_H 7.22 (1H, br s, H-21). The tertiary methyl at δ_H 1.30 (3H, s, 19-CH_3). Please correct.
    Response: I don't understand what's to be corrected here
46. Table 4.5. the assignments of carbons C8, 9, 20, and 30, are different from the reported values and changed by the candidate without discussion and justification; please re-check, discuss, and justify
    Response: The difference in chemical shift looks small to me.
47. Page 151. Compound 80, obtained as a white solid, was identified as a novel steroid. Please check WO2018187804.
    Response: WO2018187804 describes the synthesis of sulfonyl carbamate bile acid derivatives. Some intermediates compounds have structures close to compound 80 (Figures 4.16, 4.17, Page 152-153).

[EN] PROCESS FOR PREPARATION OF SULFONYL CARBAMATE BILE ACID DERIVATIVES
[FR] PROCÉDÉ DE PRÉPARATION DE DÉRIVÉS SULFONYLCARBAMATE D'ACIDES BILIAIRES







48. Page 151. and the proton at δ_H 2.03 (1H, m, H-6b) with C-8 (δ_C 50.03), C-5, C-1, and C-7 (δC 211.71). check the correlation with C-1 (it's not possible).
    Response: There is a small correlation between H-6b and C-1. It's four bond away.
49. Page 151. as well as between H-13a (δH 1.92, m). please correct.
    Response: Corrected (Page 151)
50. Page 152. and HMBC correlations between 21-CH3 and C-17, C-20 (δC 42.41), C-22 (δC 177.30) and between the methoxy (δH 3.64, s) and C-22, constructed the side chain assigned at C-17. This is not matching with the table and figure 4.16
    Response: Corrected (Page 152)
51. Page 152. The relative configuration was determined from the NOESY spectrum. **The Relative**

configuration is weakly discussed, and more evidences are required. Please use the 3D structure to indicate the configuration and support with evidence and more literature.

Response: Noted

52. Page 152. compound (80) was established as 3α-hydroxy-23-bisnorchol-7-one-22-oate. This is different from figure 4.17.
    Response: Corrected (Page 152)
53. Page 155. 7.15 (1H, s, H-21). Please correct
    Response: Corrected (Page 155)
54. Page 155. in the HMBC spectrum, the correlations between H-1 (δ_H 4.74, dd, J = 3.11, 5.25 Hz) and C-3 (δ_C 168.91), C-4 (δ_C 85.32), C-10 (δ_C 44.20) and 28-CH3 (δ_C 29.23). please check again carefully, “not possible”.
    Response: Corrected (Page 155)
55. Page 159. 1H NMR spectrum displayed two singlet signals at δH 6.00 (1H, s, H-30a). // two olefinic proton signals appearing at δH 7.46 (1H, br s, H-1) and 6.07 (1H, br s, H-2) // please correct
    Response: Corrected (Page 159)
56. Page 163. [signals at δH 7.44 (1H, s, H-23), 7.27 (1H, s, H-21), and 6.33 (1H, s, H-22)], one acetyl group [δH 2.10 (3H, s, 1'''-CH3)], a formyl proton at δH 7.82, three tertiary methyls resonating at δH 1.03 (3H, s, 18-CH3), 1.80 (3H, s, 19-CH3), and 1.85 (3H, s, 28-CH3). // between H-17 (δH 3.99, t, J = 8.67 Hz) //H-11 (δH 5.53, dd, J = 7.61, 10.21 Hz) and C-10 (δC 46.05), C-12 and C-OO (δC 160.27), H2-30 (δH 5.93 (br s) and 5.57 (br s)) // the methyls at δH 0.79. // and at δH 0.85 (3H, d, J = 6.60 Hz, 6'- CH3) and C-3', C-4', C-2' (δC 74.63), and H-2' (δH 3.12, br s). Please correct the values according to table 4.9.
    Response: Corrected (Page 163)
57. PAGE 164. Table 4.9. the C-13 chemical shifts of C-1, 2, 5, 6, 11, 12, 16, 17, 20, 28, 29, and 30 are different from the reported values. Please check, discuss and justify the right values.
    Response: The difference is negligible and stays in the limit of respective functional groups (Table 4.9, Page 164).
58. Page 177. 1-desoxy-1α-peroxy-rupicolin A 8-O-acetate, 1-desoxy-1α-peroxy-rupicolin B-8-Oacetate, rupicolin A-8-O-acetate, rupicolin B-8-O-acetate, 1,13-dehydromatricarin, 1α,4α dihydroxybishopsolicepolide and 1α,4α-8α-trihydroxyguaia-2,9,11(13)-triene-12,6α-olide-8-Oacetate. O should italic.
    Response: Corrected (Page 177)
59. Page 181. The bottom MeOH/H2O (85:15) layer was repartitioned with DCM (3 × 500 mL). this part needs careful revision, addition of DCM to the aq. MeOH will absorb all the MeOH and leave small layer of H2O
    Response: The layers were well separated (Page 181)
60. Page 184. 7,3’,4’-tri-O-methylluteolin (95), and 7-O-methylacacetin (84). O is italic.
    Response: Corrected (Page 184)
61. Page 186. Single crystals of 7-O-methylacacetin (84). O is italic.
    Response: Corrected (Page 186).
62. Page 197. two tertiary methyls at δH 1.33 (3H, s, 14-CH3) and 1.61 (3H, s, 15- CH3). Please use the right description
    Response: Corrected. Tertiary removed (Page 197).

63. Table 5.4. the C-13 chemical shift values of C-2, and -8 are different from the report ones. Need verification and discussion
    Response: The difference is negligible. (26 vs 27 and 28 vs 26, seems acceptable). Table 5.4, Page 198.
64. Page 199. δC 118.81 (C-13) and in the HMBC spectrum with C-6 (δC 80.84), C-7 (δC 52.36), C-11 (δC 139.92), C-12 (δC 170.52). // δC 91.78 (C-1). correct according to table 5.5.
    Response: Corrected (Page 199)
65. Page 201. m/z 247.1378. italic.
    Response: Corrected (Page 201)
66. Page 203. 5.52 (1H, m, H-15), 5.39 (1H, m, H-16). Check and correct.
    Response: Corrected (Page 203)
67. Page 207. at m/z 299.0915. italic
    Response: Corrected (Page 207)
68. Table 5.10. please check the chemical shift of C-9
    Response: It is correct (Table 5.10, Page 211)
69. Table 5.14. please add positive control.
    Response: Added (Table 5.10, Page 218)
70. Chapter 6. Please add few line to cover the future recommendations
    Response: Added