Author's response to reviews

Title: Antagonistic effect of alkaloids and saponins on bioactivity in the quinine tree (Rauvolfia caffra Sond.): Further evidence to support biotechnology in traditional medicinal plants

Authors:

Trizah K Milugo (tesskoyi190@yahoo.com)
Leonidah Kerubo Omosa (lkerubo@uonbi.ac.ke)
Bethwell O Owuor (owuorb2001@yahoo.com)
James O Ochanda (jochanda@uonbi.ac.ke)
Joel W Ochieng (jochieng@uonbi.ac.ke)

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Author's response to reviews: see over
Dear Editor,

RE: SUBMISSION OF REVISED MANUSCRIPT (R1)

We submit a revised version of the manuscript entitled “Antagonistic effect of alkaloids and saponins on bioactivity in the quinine tree (R. caffra Sond.): Further evidence to support Biotechnology in traditional medicinal plants”, by Milugo et al.

Review comments received were very useful in improving the science, communication, and in making the manuscript a better read. This manuscript is now a valuable resource for phytomedicine and associated biosafety assessments.

Specific comments by the reviewers were considered carefully and mostly adopted, and the very few that could not be changed without altering the context or intended meaning are indicated in the section below.

Yours Sincerely,

Joel W. Ochieng, PhD
Corresponding Author


Reviewer: SHAIVAL KAMALAKSHA RAO

Sample collection, storage and treatment
1. The authors should mention the authenticity of the sample by giving details about: Who authenticated the sample?, Whether a Herbarium Specimen was made?, and collection time of the plant material.

Text now added to indicate: “Samples were collected in March 2013 and authenticated by a botanist, Professor J.O. Kokwaro. Herbarium specimens were deposited at the University of Nairobi herbarium, under specimen Voucher No: Mutiso-RC-23/3/2012”.

2. For TLC, the ratio of Solvent system used should be mentioned clearly.

Statement no reads: “…..followed by a combination of DCM/Methanol (97:3 v/v) and finally DCM/Methanol (19: 1 v/v)…..”.

3. For DPPH assay, what reference was followed?

Citation has been added to that section (Hatano et al, 1988), and this has been reflected in the list of references.

4. The authors are truthful about their findings, but, little more has to be established before publication of the manuscript. What are the compounds responsible for the Antagonistic action? Can this antagonistic action be terminated by any means?

It is now clarified that the presence of both alkaloids and saponins is responsible for the antagonistic action. It is clarified in a different section that biotechniques can be used to terminate this action.

5. The authors need to look into the possible mechanism of action of effectively killing of tumors as per claims of traditional healers.

We are sourcing funds to perform tests of extracts on cancer cell lines

Quality of written English: Needs some language corrections before being Published
Language now greatly improved in this version, buoyed by attending to all review comments and authors efforts

Reviewer: SHAMIM QURESHI

Major Compulsory Revisions: None

Minor Essential Revisions:
1. Abstract: rephrase the conclusion portion
2. Introduction: i. It requires thorough rephrasing in order to remove repetitions and make it precise and meaningful. In its present state, many sentences deliver the same concept in different words that would put burden on readers' minds. ii. Author is just suggesting that genetic manipulation could enhance the bioactivity of R.caffra without doing any experiment related to it that's why kindly make this concept precise, don't make it lengthy. iii. Author has written aim of study first at the end of fourth paragraph then in the last paragraph, kindly write it collectively or in improved way in the last paragraph so it could be more powerful and dominating.

The sentence “This study aimed at identifying a plant species from which strong antioxidants and other phytochemicals with health benefits can be produced, and where metabolic pathway engineering can be employed to design optimal production of desired phytochemicals” has been deleted and enhanced, the last paragraph enhanced to read “This study aimed to identify available phytochemicals in *R. caffra* with known function in human health, and to test individual and collective bioactivity of these compounds”.

3. Methods: i. TLC assay: No reference is mentioned in determining the antioxidant activity on TLC plate, kindly add it. ii. DPPH free-radical scavenging assay: Kindly add reference which states that whose method you have cited. iii. Statistical analysis: write it in separate sub-heading.

    (i) TLC: Citation has been added to that section (Hatano et al, 1988), and this has been reflected in the list of references.
    (ii) DPPH: Citation has been added to that section (Mongkolsilp et al, 2004), and this has been reflected in the list of references.
    (iii) Implemented – subheadings for statistical analysis included

4. Results and discussion: i. Write results and discussion in separate heads (check recent papers published in BMC CAM), it would remove sub-headings in discussion which are not normally present in this section. ii. Interestingly, discussion has many sentences which are already written in introduction, kindly take care of all these repetitions.

Repetitive sentences have been deleted. Subheadings are necessary to lead/guide the reader, and have been toned down and edited according to the other reviewer comments. I suppose from the instructions that the Journal allows for combination of these two sections. However, reviewer or editorial decision to remove subheadings at typesetting stage will be ok.

5. Conclusion: Kindly improve it.
Conclusion now shortened and focused. Unnecessary sentences purged.

6. References: i. These are mentioned in numeric figures within block brackets in text but in reference section no number is mentioned in front of each reference. Improve it.

Implemented – References now numbered

Quality of written English: Needs some language corrections before being Published

Language greatly improved with revisions of the sections

Reviewer: MEHRAN FADAEINASAB

Reviewer’s report:
Major Compulsory Revisions
It could be better if the authors isolate some pure compounds from this plant and identify them by using spectroscopic methods such as NMR, Mass, IR and UV. the authors could run some other activities like anti cancer which is common for this plant’s family due to their toxicity of the compounds

This suggestion is helpful but the current study was exploratory in nature, aimed at first establishing the level of antioxidant activity of various classes of compounds, and in this instance reports on the effects of simultaneous presence of two antagonistic phytochemicals. We are currently sourcing funds to expand into toxicity tests, first using cancer cell lines and model organisms (rats).

Quality of written English: Acceptable

Reviewer: PRASANNA KUMAR SP

Reviewer’s report:
Major Compulsory Revisions
The Comments are
In Title
Please use the word Antioxidant in the title of the paper.

Bioactivity includes antioxidant activity. We expect that this antagonistic relationship may affect other biological activities, and that antioxidant activity is only the manifested element exhibited by the methods/tools used in this study. Can be altered if this is ok.
In Introduction
Mention the current status of Rauvolfia caffra in Red data book.

Sentence added to read: “This species is still of less conservation concern in many countries, such as South Africa (Fodden and Potter, 2005).”

Gene modification of wild plant species and its release again into the World is not acceptable even though it is self pollinated plant. It can only grow in controlled condition and the bioactive molecules can be extracted and used for medicinal purpose, hence these lines from the introduction part as it is not relevant for yours study.

Sentence now reads: “Although *R. caffra* is a sparsely populated remnant tree species that is purely selfing, hence the threats of gene flow is limited, it should be domesticated under controlled conditions where the bioactive molecules can then be extracted and used for medicinal purpose.”

In Methodology
Reference should be given for solvent extraction procedures.

Reference added to the text in this section (Sengul et al, 2009) and included in reference list.

Mention the % of active ingredients of commercially used drug Quercetin in this study.

Quercetin (3,3’,4’,5,7-Penthydroxyflavone) is a polyphenolic bioflavonoid. It is the the bioflavonoid that is the active. This is now clarified in the text where it is first mentioned.

Results and Discussion
Even though the results are informative, the data presented is insufficient, the authors are not quantified the level of Saponins and Alkaloids in the extracts. The solvent system which contains the activity is not clear. From the Table 2, stem bark and leaves contain almost equal amounts of Alkaloids and Saponins, but in the results which is stated that stem bark having more antioxidant activity. Specify?

Antioxidant activity does not only result from presence of the two phytochemicals – apart from alkaloids and saponin, we detected terpenoids, steroids, and cardiac glycosides. So stem bark and leaves could contain equal amounts of the first two, but differ in their content for the other compounds (for example see terpenoids in Table 2) – hence the difference in antioxidant activity.

From the Table 3, fraction FN which contains all alkaloids and Saponins showed 58% inhibition and fraction FF which contains only alkaloids and showed 62%
inhibition. It is not correlating with your explanation, where Alkaloids and Saponins were found to be suppressing antioxidants activity.

Table 3 shows the following:

1. FN: All phytochemicals detected = 58.9%
2. FM: All minus saponin = 82%
3. FF: Alkaloid only = 62%
4. FB+C: Alkaloid and Saponin = 15%

Fraction FN contains all phytochemicals detected, not just alkaloids and saponins.

Add statistical values for the mentioned significant fractions.

Percentage inhibition for all fractions were significantly different from each other (p = 0.027). This has been added to the text.

References
Add serial numbers to all the references according to the reference cited in the content of the paper.

All references now numbered according to appearance in text

Quality of written English: Needs some language corrections before being Published

These have now been done and the manuscript is an easier read

Reviewer: SIMON KWAKU ATTAH

Reviewer’s report
DISCRETIONARY REVISIONS
Title: This should be framed as: “Antagonistic effect of alkaloids and saponins from the quinine tree (Rauvolvia caffra Sond.) on bioactivity: Further evidence to support biotechnology in traditional medicinal plants.”

Not implemented – this would change the intended meaning. It was a discretionary suggestion anyway.

MINOR ESSENTIAL REVISIONS
ABSTRACT:
Background:
Ln1 “Quinine tree” should read as “the quinine tree”. This should be corrected wherever this appears in the manuscript.

Implemented in abstract and every necessary place throughout the manuscript
Methods:
Ln 1: DPPH should be written in full and the abbreviation placed within parenthesis.

That section now showing: 1,1-diphenyl-2-picrylhydrazyl (DPPH) as recommended

Conclusion:
State clearly the conclusion of the study and what is stated in the abstract should correspond with what is stated under the discussion.

Last sentence of conclusion deleted and the others rephrased to be concise and straightforward; also following other reviewers’ comments

Key words: the semicolon (;) placed after the “Key words”, should be changed to a colon (:)

Implemented

BACKGROUND:
Para. 1, Ln 5: the statement: “making many to turn to …..” should read as: “thereby making many to turn to ……..”

Implemented

Para. 2, Ln 2: “……traditional prescription of crude extract is unsuitable for two reasons:” should read as follows: “……the traditional prescription of crude extracts is unsuitable for two reasons:”

Implemented

Para. 2, Ln 9: the statement: “thus affecting the levels of abundance for the bioactive compounds” should read as: “thus affecting the levels of abundance for the bioactive compounds.”

The period should appear after the reference as in the text. Version retained.

Para. 3, Ln 4: the statement should read as follows: “Although genetic manipulation (GM) is a controversial subject, stronger objection concerning food crops, where GM activities…….. “

Implemented

METHOD:
Sample collection, storage and treatment:
Para. 2, Ln 4: insert a comma after “finally” and insert a reference(s) at the end of
the statement.

Comma added; reference (Sengul, 2009) added

Thin layer chromatography assay:
Para. 1, Ln 2: the statement should read as follows: “This method was used to
detect the presence of compounds in the crude extracts.”
Implemented

Para. 1, Ln 5: the phrase: “…..and finally in a combination with 5% Methanol.”
Should read “…..and finally in a combination where Methanol is 5%.”

This has been revised as recommended by another reviewer, and now reads: “….and
finally DCM/Methanol (19: 1)”.

Fractionation of phytochemicals in stem bark crude extract:
Para. 1, Ln 11: The statement: “The phytochemicals probed were; flavonoids,
coumarins, alkaloids, steroids, cardiac glycosides, saponin, terpenoids, tannins
and phenols.” Should read as: “The phytochemicals probed were: flavonoids,
coumarins, alkaloids, steroids, cardiac glycosides, saponins, terpenoids, tannins
and phenols.”
Implemented – full colon has replaced the semi-colon

RESULTS AND DISCUSSION:
Para. 1 Ln 4: “Presence of such phytochemicals…………” should read as: “The
presence of such phytochemicals…………”
Implemented

Para. 1, Ln 7: This statement should read: “We found R. caffra to be rich in
antioxidants, and contain several known phytochemicals, two of which showed
antagonistic effect.”
Implemented

Sub-heading: “Quinine tree was a strong antioxidant” should read as: “Quinine
tree as a rich antioxidant plant.”
Implemented

Para. 1, Ln8: a comma (,) should be placed after (Indeed) in the statement as
follows: “Indeed, it is likely that R. caffra antioxidant activity is stronger than
quercetin,.........”
The subheading: “Alkaloids and saponins showed antagonistic bioactivity” should read as: “Antagonistic bioactivity of alkaloids and saponins”

Para. 1, Ln 12 In the statement: “Saponins observed in this study are likely to be steroids,...........” What makes the authors think so? Could you explain?

It was explained that “Saponins observed in this study are likely to be steroids, the type common in wild plants used as herbs, while those that occur in cultivated crops are predominantly the triterpenoid saponins; and Fenwick et al 1991 is cited.

Sub-heading: “Modern biotechnology can optimize medicinal value of R. caffra” This should read as: “Modern biotechnology and the optimization of medicinal value of R. caffra.”

CONCLUSION:
Para. 1, Ln 3: the word: “extract” should read “extracts”

NB: The conclusion should be brief and straight to the point.

Conclusion now shortened and focused, also as advised by the other reviewers

REFERENCES:
Ref. 3: 9(49): 8467-8471 should be read as: 9(49):8467-8471


NB: References should be numbered accordingly.

All references now numbered

Quality of written English: Acceptable

Accept after minor essential revisions (which the authors can be trusted to make)