Author's response to reviews

Title: Evaluation of antioxidant and anti proliferative activity of Flueggea leucopyrus Willd. (katupila)

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Author's response to reviews:

Dear Mr Prozenko

Thank you for extending the dead line for couple of days. Till the last moment I was trying to meet the dead line. Some of the experiments were also done to get the article published.

Reasons for delay,

• I wanted to do the MTT assay for 72 hours with HEp 2 cell line, but unfortunately I could not find it in our laboratory and had to get from another laboratory and still it is growing.

• Repeated some experiments for antioxidant activity for positive control to improve the quality of the article.

The plant Flueggea leucopyrus is recently got very popular among cancer patients, and growing this plant in their back yard. This is an attempt to explore its scientific value.

Following are the revisions for the reviewers comments.

Reviewer: Gerhard Prinsloo

Minor essential revisions:

Comment 1.

F. leucopyrus can be used throughout and not the complete scientific name

Revision

Flueggea leucopyrus was replaced by F. leucopyrus as suggested
Comment 2. Correct spelling for "Folin Ciocalteu" in abstract

Revision
It is corrected in the manuscript

Comment 3.
Last sentence of introduction, a full stop has been left out - "therapeutic efficacy As a result"

It is corrected

Comment 4. Materials and methods - carbon dioxide or CO2 to be used consequently

It is corrected

Comment 5. First sentence in discussion - remove the in the sentence "are the secondary metabolites in" as they are only some of the secondary metabolites.

Revision
Word ‘the’ is removed as instructed by the reviewer.

Comment 6. Discussion - The sentence seems to be out of place as it speaks to animal defence - "are nature’s gift to animals to protect them".

Revision
Sentence was replaced by ‘Phenolic compounds also provide us natural antioxidants for protection against many diseases’

Figure 1 - Cytotoxicity incorrectly spelled.

Revision
It is corrected in the figure axis

Comment 8. Results antioxidant activity - provide the values for ascorbic acid.
The table was reconstructed inserting the EC50 values for positive controls.

Comment 9. Results MTT assay - which positive control used?
Camptothecin (5mM, 20µL) was used. It is inserted in the methodology as well as the results.

Comment 10. Materials and methods - morphological changes. Insert full stop "presented in Figures 3 The untreated"
It is corrected in the revised manuscript.

Comment 11. Check spelling for camptothesin - morphological changes.
It is corrected in the revised manuscript.

Quality of English
English was corrected and sent to Professor Ramani Wijesekera for editing the manuscript.
In the acknowledgement her name was added.

Reviewer: Dr. N Kishore

Major revisions;
Regarding EC50 values for 72 hours
The present study was not carried out until 72 hours. I did want to repeat it for 72 hours according to Dr Kishore’s suggestion. At present it was not available in our lab.

However 30 #g/mL for EC50 is hypothetical value. This value depends on the number of cells we are using. eg According to my experience same EC 50 value may not be observed if we use different well plates (8 well, 12 well, 24 well and 96 well, cell culture plates).

However in this study it is very clearly observed apoptotic cells and apoptotic bodies when stained with AO/EB. DNA fragmentation confirmed it.

Comment: `Significant antioxidant activity`-

Revision
Minor essential revisions

Comment

There are others secondary metabolites including many alkaloids have been reported previously from this plant species but author didn’t mention in the plant description in introduction section instead mentioned compounds from other species. Why?

Revision

Included the following two sentences in the revised manuscript

`More than ten secondary metabolites are identified in F.leucopyrus (7, 8). One of the major active constituent found in methanol-water (80:20) extract of the F.leucopyrus leaves is bergenin and it has shown antioxidant and immunomodulatory activities in vitro (8).`

Comment

Positive control for antioxidant assay and MTT assay has been used but the values of positive control in comparison with plant extract are unclear and not written correctly in the tables.

Revision

Positive control (camptothecin) showed 76.07±1.72% growth inhibition at the concentration (5mM, 20µL) used. This is included in the Table 2 as instructed.

Comment

Authors used short form of many words, so there should be a list of abbreviation for all the abbreviated forms used in the manuscript.

Revision

Abbreviations

AEFLL: Aqueous extract of F. leucopyrus leaves
AO/EB: Acridine orange (AO)/ ethidium bromide (EB)
GAE: Gallic acid equivalents
DPPH: 1-Diphenyl-2-picrylhydrazyl
MTT: (3, 4, 5-(dimethylthiazol-2-yl) 2-5-diphenyl tetrazolium bromide)
LDH: Lactate dehydrogenase

The changes in the manuscript
Instead of the last paragraph, a new paragraph of conclusion was included to make the summary and conclusion more clear in the revised manuscript as follows.

Conclusion
F. leucopyra is considered as a plant containing anticancer activity and the water extract of leaf is consumed as a dietary supplement. The high antioxidant activity and phenolic content shown by the aqueous extract of the plant suggest that it is a potential therapeutic agent for the control of oxidative damage caused by reactive oxygen species and especially nitrogen species. F. leucopyra showed DNA fragmentation in HEp2 cells even after 24 hour exposure of the leaf extract indicating its ability to induce apoptosis. This study provides the scientific proof of the traditional knowledge in using the leaf extract as an anticancer agent.

Table 1 and Table 2 were reconstructed as suggested by reviewers

Table 1: EC50 values for DPPH, NO and hydroxyl radical scavenging activity of the AEFLL (n=3) and respective positive controls.
Radical scavenging assay EC50 (µg/mL)
Plant extract Ascorbic acid Gallic acid
DPPH 11.16±0.45 4.28±0.32 -
NO 4.82±1.82 54.26±6.54 -
Hydroxyl radical mediated 2- deoxy-D-ribose degradation 23.77±3.87 - 8.27±0.53

Table 2 The EC50 values obtained for MTT, LDH and brine shrimp assays for AEFLL and Percentage inhibition of cell growth by camptothecin (5mM, 20µL)
Cytotoxicity Assay EC50 value (µg/mL) % inhibition by camptothecin (5mM, 20µL)
MTT (n=4) 506.80± 72.93 76.07±1.72%
LDH (n=4) 254.52±42.92 50.51±7.67%
Brine shrimp (n=3) >500