RESPONSE TO THE REVIEWERS' COMMENTS

Editor

Comments	**Response**
Main comment: How do the authors explain the variability in response to FRAP analysis (Figure 3A-C)? This reviewer understand that the extract can have a maximum effect at 1 ug/mL, but how is possible that from that concentration the antioxidant effect varies randomly? Was all the range of concentrations performed at the same time? Please explain.	Yes, the cell culture assays were performed in parallel for each treatment at all range of concentrations. However, results showed that the samples reacted in a dose-specific manner instead of a dose-dependent manner, as described on page 13, lines 289-295. This phenomenon could be due to lack of significant effect at lower concentrations, meanwhile higher concentrations could have caused xenobiotic stress, as described on page 13, lines 302-305.
Minor comments: P8L167 please indicate the plate format used P9L195 please indicate the plate format used	P8L173: plate format has been inserted P9L202: plate format has been inserted
Synergic effect of polyphenols have been previously described in the literature, unless it is experimentally shown in your results please reduce the tone by using "might" or "could" to point this possibility (P13L289).	The change has been made as in P13L294

Reviewer #1

Comments	**Response**
Basic reporting The article fulfills the standards required. The work is clearly written and fits into the broader field of knowledge. Just some minor revisions: Page 1, Line 70 : Consider changing the phrase to "Hepatocellular carcinoma cells, HepG2, are a well established cell line"	The phrase has been changed as suggested, in P3L71.
Page 1, Line 72: Phenolic acids and flavonoids from plants are metabolised by	The phrase has been changed as suggested, in P4L73.

the liver after absorption, mainly, in the small intestine.	
Experimental design The article fullfills the standards required. The primary research is original and fits within the scope of the journal. Methodology seems correct and reproducible.	We thank the reviewer for the positive feedback.
Validity of the findings The article fulfills the standards required. Data seems solid and statistically correct. Conclusions are appropriately stated, connected to the original question investigated, and are limited to those supported by the results.	We thank the reviewer for the positive feedback.

Reviewer #2

Comments	**Response**
Basic reporting Title: Concise and reflect the content of the article. Suggestions: The authors work with extracts and should be reflected in the title.	The title has been changed to include the word 'extracts' as suggested.
Abstract: It is brief and describes clearly the purpose of the work and the major results.	We thank the reviewer for the positive feedback.
Introduction: This section is clear and well organised. Reflect the importance of using natural antioxidants to balance the oxidative stress to avoid aging and degenerative diseases. Specific improvements: - L57 – Exogenous and endogenous antioxidants can limit or reduce the oxidative stress but no its elimination. Please, revise this paragraph.	P3L57: The word "eliminating" has been replaced with "reducing"
Tables and figures: Comments and corrections:	Figure 2 has been removed and replaced with Table 1 as suggested.

Figure 2. These data could be more conclusive represented in a table where it will be easily to show the sample concentrations and the % of viability.	
References: The reference section is appropriate and updated. Corrections: - L430 – Correct the surname of the author: D'Archivio	The surname has been corrected, P19L436
- L474 – This reference does not appear in the main manuscript.	The reference has been removed, P21L480.
Experimental design Material and methods: Properly describes the methodology used. Comments and corrections: - L84 – Please, insert the nationality of the supply company	The required information has been inserted, P4L86.
- L99 – Why do the authors subject the lyophilised extracts to acid hydrolysis? Please, explain it in this section. Did the authors analyse the samples without previous hydrolysis? The hydrolysis could degrade some polyphenols present in the extracts and the authors do not identify them but they are present in the in vitro assay.	For the purpose of cell culture treatment, only lyophilised extracts were utilised. However, for analysis and identification of polyphenols in the extracts using HPLC-DAD, the samples were subjected to acid hydrolysis. Polyphenols in plants exist either in the free or bound forms (as glycosides). Hydrolysis helps to release the free polyphenols (aglycone) from the glycosides. This provides a practical approach towards the identification of polyphenols through HPLC analysis. We have included a short write-up in the manuscript to clarify this, see P5L105-107. Optimisation of this method including recovery has been described by Hertog et al., (1992): Michael G. L. Hertog, Peter C. H. Hollman, and Dini P. Venema. Optimization of a Quantitative HPLC Determination of Potentially Anticarcinogenic Flavonoids in Vegetables

	and Fruits. *J. Agric. Food Chem.* 1992, *40*, 1591-1598
- L101 – 90ºC - L107 – 30ºC	The corrections have been made in the manuscript.
- L110 – Please, substitute diode array detector for DAD.	Diode array has been substituted with DAD in P5L114.
- L110 – Detection at 280 nm is most commonly used for phenolics acids, although monitoring at 254 and 320 nm can provide more information about other polyphenols presents in the samples. Why do the authors use only the 280 nm wavelength?	In our preliminary studies (Kong et al. 2012, Food Chemistry; Kong et al., 2014, Food Chemistry), the detection of polyphenols in *B. racemosa* was performed at different wavelengths, including 254, 280 and 325 nm. However, overall, 280 nm provided a better detection for polyphenolic compounds in this plant.
- L127 – 37ºC	The correction has been made in the manuscript.
- L135 – MTT reagent was dissolve in?	The details have been added in P6L138.
- L146 – Why do the authors use these concentrations of gallic acid? No quantification of phenolic compounds in the samples was done.	The concentration of gallic acid was determined based on the total polyphenolic content reported in our previously published paper (Kong et al. 2012). We have included a short write-up in the manuscript to clarify this. See P7L151.
- L150 – 37ºC	The correction has been made in the manuscript.
- L151 – Please, insert the absorbance reader specifications.	The details of the spectrophotometer and fluorescence spectrophotometer have been included, P6L140 and P8L179.
- L163 – Please, specify the expression of the results.	The details have been added in P8L168.
- L167 – Please, indicate the format of plate used for to seed the cells.	The details have been added, P8L173.
- L174 - Please, specify the expression of the results.	The details have been added, P8L181.

- L187 – 25 N	P9L194: The correction has been made in the manuscript.
- L188 - 37ºC	The correction has been made in the manuscript.
- L195 - Please, indicate the format of plate used for to seed the cells.	The details have been added, P9L202.
Validity of the findings Results and discussion: The presentation of the results and the discussion is clear, well-structured and follows a logical sequence.	We thank the reviewer for finding the results and discussion clear and well-structured.
Comments for the author PeerJ Manuscript Number: Peerj-6893 Title: "Protective effects of Barringtonia racemosa shoots against oxidative damage in HepG2 cells" Author (s): Kin Weng Kong, Sarni Mat-Junit, Norhaniza Aminudin, Fouad Abdulrahman Hassan, Amin Ismail, Azlina Abdul-Aziz General comments The search for naturally occurring compounds with beneficial health effects that can be used as potential food ingredients has a great interest. Therefore, the researches trying to characterize the potential active compounds from natural source and to associate some beneficial effects (antioxidant, anti-inflammatory, antitumor, antibacterial, etc) are very promising. This paper attempts to characterize natural plant extracts and associate them with certain beneficial properties such as its potential antioxidant capacity. The authors have recently studied the antioxidant activities of Barringtonia racemosa shoots (Kong et al., 2012 and 2014) as well as other authors (Razab et al., 2010), but in this novel work, the authors try to proof the	We thank the reviewer for finding the work to be novel and of potential interest to the scientific community. Thank you.

antioxidant activity in vitro using human cells. Therefore, the objective addressed in the present article can be considered of great interest and meets the requirements for scientific publication.	