



**SDI Review Form 1.6**

Journal Name:	<a href="#">Asian Food Science Journal</a>
Manuscript Number:	Ms_AFSJ_48607
Title of the Manuscript:	COMPARATIVE STUDIES ON ANTI-INFLAMMATORY, ANTIOXIDANT and ANTIMUTAGENIC ACTIVITIES of <i>Crassocephalum crepidioides</i> (Bent) LEAF COLD AND HOT WATER EXTRACTS
Type of the Article	Original Research Article

**General guideline for Peer Review process:**

This journal's peer review policy states that **NO** manuscript should be rejected only on the basis of '**lack of Novelty**', provided the manuscript is scientifically robust and technically sound. To know the complete guideline for Peer Review process, reviewers are requested to visit this link:

(<http://www.sciencedomain.org/page.php?id=sdi-general-editorial-policy#Peer-Review-Guideline>)



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**PART 1: Review Comments**

	Reviewer's comment	Author's comment (if agreed with reviewer, correct the manuscript and highlight that part in the manuscript. It is mandatory that authors should write his/her feedback here)
<b>Compulsory</b> REVISION comments	<p><b>Comments on COMPARATIVE STUDIES ON ANTI-INFLAMMATORY, ANTIOXIDANT and ANTIMUTAGENIC ACTIVITIES of <i>Crassocephalum crepidioides</i> (Bent) LEAF COLD AND HOT WATER EXTRACTS</b></p> <p>General comments Author(s) of the current manuscript try to show the anti-inflammatory, antioxidant and antimutagenic activities from two kinds of extracts obtained from <i>Crassocephalum crepidioides</i>. Although the methods are in concordance with the objective of the study, the extraction methods reported are not described correctly in order to be reproducible and several details should be clarify. Besides, even that selected biomarkers for antioxidant response are adequate, its presentation and interpretation is not clear. Due that inflammatory process is complex, the use of one biomarker (Membrane Stabilizing Potential) is not enough to affirm or deny the anti-inflammatory properties of the extracts. This method should be complemented with an in vivo model. Why onion was selected as a model for genotoxicity? At least another mutagenicity test should be done to properly assess the mutagenicity of the extracts. There is a lack of chemical characterization of the extracts, total polyphenols and flavonoids are not enough. The graphs are not easy to read, and several questions arise from its observation. Although, half maximal inhibitory concentration (IC<sub>50</sub>) is an important variable in toxicology studies, for these results maybe is more easy and practical to represents as percentage of positive or negative control. Even bar graphs could be more suitable. The results sections is excessive in terms of size, please be more concise and clear. The discussion section not allow to compare the results of the study with other published with plant extracts. Are these extracts more, equal or less beneficial than other obtained from other plants? What chemical species in the plant extracts exert the biological effects observed in the investigation? What mechanisms are involved in these biological processes?</p> <p>Particular comments Introduction: Line 46-47: This sentence is kind of excessive just clarify that "Excessive activation of phagocytes and reactive oxygen species (ROS) are..." Line 54-55: "Herbal compounds" Line 56: For first time in the manuscript you should mentioned the complete scientific name of the plant under study "<i>Crassocephalum crepidioides</i>" and then, through the entire manuscript as "<i>C. crepidioides</i>". I also recommend including the common names of this plant between parentheses due that some plants are called with different names in different places of the world. Line 58: Erase "y" Line 58 and 59: Maybe for the objective of the investigation is irrelevant to mention the different ways in which the leaves are prepared for be eaten in different localities such as Sierra Leona or Australia. However, is more important remark that the leaves could be eaten cooked or raw in different parts of the world and this is the justification for the use of cold and hot water extracts in this research. Materials and method: Line 69: Although is mentioned that <i>C. credipioides</i> were collected from Ile-Ife, it is not clear if these vegetables were bought from a local marker or supermarket, or if were wild or obtained from a crop. This is relevant because the contamination is not the same depending of the source, for example, is more expected that the concentration of pesticides were greater in vegetables from a crop in comparison with the obtained in wild.</p>	<p>Yes sir. The manuscript described the anti-inflammatory, antioxidant and antimutagenic activities of two kinds of extracts obtained from <i>Crassocephalum crepidioides</i>. The presentation and interpretation of anti-oxidant results have been clearly presented. The extraction methods reported have been described correctly in order to be reproducible.</p> <p>Inflammation is indeed a complex process. However, irrespective of the etiology, cell membrane disruption is usually a general mechanism in all forms of inflammation. Thus disruption of cell membrane is considered the fundamental basis of inflammation. This explains why membrane stabilization technique is accepted globally for evaluating extracts with anti-inflammatory activity. <i>Allium cepa</i> model is a globally accepted technique for investigating compound(s) with genotoxic property. There is lack of chemical characterization of the extracts because that is not what the study was designed to achieve. The essence of the study was to establish the scientific basis for the use of the plant in ethnomedicine. The graphs are readable sir. It is just a plot of percentage stability against the concentrations of extracts. The various behaviours of the extracts are interpreted as biphasic depicting presence of different constituents that may be antagonistic in effect. IC<sub>50</sub> is also a standard way for representing DPPH, FRAP, TBARS in standard literatures. Besides, the use of IC<sub>50</sub> made it easy to define potency.</p> <p>Sir, this is a full length article. Result section is not excessive. The discussion section has been compared with relevant works. The study suspected pyrrolizidine alkaloids –chemical species reported to be present in the plant species and with genotoxic property. Mechanisms have been succinctly described. <b>Sir, the work is a Novel work.</b> The manuscript is scientifically robust and technically sound. I maintain that the work is suitable for publication. Why hiding this information from the world? Line 46-47: sentence has been modified.</p> <p>Line 54-55: changes have been made sir. Line 56: the correction has been effected. Thank you sir. Common name in Yoruba Southwestern Nigeria (where the vegetables are extensively used) has been effected.</p> <p>Line 58: "y" has been erased sir. Line 58 and 59: thank you so very much sir for that key observation. I have also modified that statement by removing the different ways in which the leaves are prepared in different Sierra Leona and Australia. However, the forms in which the leaves are eaten around the world (cooked or raw) is</p>



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	<p>Line 74-80: What were the conditions of the blending process such as the model or label of the blender or the time? Provide enough information for the reproducibility of data. Other concern with this section is that “the homogenates were filtered with a double layered cheese cloth”, how to reproduce this by others?</p> <p>Line 85: Clarify all the tested concentration of the extracts and not only its interval.</p> <p>Line 88-89: Express this formula in adequate way, not as a text. If not possible, just express “the % membrane stability was calculated according to (8)”. This also applies for lines 112, 138 and 154.</p> <p>Line 96: Which was the interval of concentration (or the concentrations) of quercetin for the standard curve?</p> <p>Line 120: Which was the interval of concentration (or the concentrations) of ascorbic acid for the standard curve?</p> <p>Line 130-131: Clarify all the tested concentration of the extracts and not only its interval.</p> <p>Line 141: Twenty five healthy onion (<i>Allium cepa</i>) bulbs... why do you use onions as model of genotoxicity and growth inhibition?</p> <p>Results:</p> <p>Line 160: The reason why diclofenac was used as control should be addressed in material and methods section. The concentrations of diclofenac should be included.</p> <p>Line 161: I cannot see the dose-dependent effect in the figure 1.0. In fact, the response showed great variability between cold and hot water extract as well as by diclofenac. I do not understand why the concentrations of diclofenac (0.5, 1, 1.5 and 2 mg/ml) appear at the same level of the concentrations of extracts (0-300 mg/ml) in the graphs. Authors must make this clear.</p> <p>Line 178: Add “mean ± SEM” before closing the parentheses.</p> <p>Line 193: Erase 0.00, just put 0.</p> <p>Sections 3.2–3.6 could be just one and named as Biochemical Assays.</p> <p>The numeration of figures is not progressive; there are two figures 1 and two figures 2. Since you report in the table 2 and 3 the effects of cold and hot water extract on mitotic index of <i>A. cepa</i> the micrographs are not necessary. Arrows and letters in micrographs should be grouped and well edited.</p> <p>What does it means “juice” in the title of figure 2 “Figure 2: The effects of hot water juice extract on the <i>A. cepa</i> chromosomes.”? Authors must clarify that the extract was obtained from <i>C. crepidioides</i>.</p> <p>Line 247: Use HWE to hot water extract and CWE for cold water extract. In fact, you can use these initials in the entire manuscript.</p> <p>Line 249-251: Any possible mechanism involved in this? Any antecedent similar with extracts of other plants? References are necessary.</p> <p>Line 252-256: This information is not valuable for discussion section. Just discuss the biological meaning of the findings.</p> <p>Line 259-260: Which kind of phytochemical species exhibits free radicals scavenging properties? References are necessary.</p> <p>Line 263-266: How do you explain these results? Why the water temperature extracts is a factor in the reductive potential of the extracts.</p> <p>Line 278-281: Information of the principle of TBARS technique is not relevant for discussion section; the important point is to discuss the biological meaning of the findings.</p>	<p>maintained. I am grateful.</p> <p>Line 69: The vegetable was collected from a local farm in Ile-Ife, Nigeria. Yes sir; the level of contamination may be higher with crop than with wild collection.</p> <p>Line 74-80: the blender model has been inserted. Filtration and other matter of concern has been addressed. The extraction method can easily be reproduced; the method is not ambiguous.</p> <p>Line 85: the extract concentrations used have been clarified.</p> <p>Line 88-89: the formulae have been addressed using the pattern suggested by you.</p> <p>Line 96: the concentrations of the quercetin used have been inserted. Line 120: it has been effected.</p> <p>Line 130-131: tested concentration has been clarified.</p> <p>Line 141: it is a globally accepted <i>in vivo</i> technique for investigating the genotoxicity potential of chemical agent or herbal preparation.</p> <p>Line 160: the reason has been effected in materials and methods. Line 161: the effect of the membrane stability is biphasic. In other words, the extract does not have a regular pattern of protection. It is not dose dependent sir. Yes sir. Both the cold water and hot water extracts displayed those patterns of activity to show that the phytochemical compositions responsible for their activity are different (perhaps) as a result of the extraction methods (cold extraction and hot extraction). Regarding the issue bordering the concentrations of diclofenac (0.5, 1.0, 1.5 and 2.0 mg/ml) and the concentrations of extracts (0-300 mg/ml). The 0.5, 1.0, 1.5, 2.0 mg/ml are independent stock solutions prepared for all the tested materials (CW, HW and diclofenac). Each of these stock solutions was varied to lesser concentrations (from 0 to 300 µg/ml) to enable us find the best concentration that would protect the erythrocytes membrane better. For example, at 2.0 mg/ml stock, the HW extract protected best while the CW did not offer any protection.</p> <p>Line 178: has been effected Line 193: 0.00 has been changed to 0. Sections 3.2–3.6 have been changed to Antioxidant Assays and the numeration has been addressed.</p> <p>Thank you sir. The purpose of the micrographs is different. The micrograph enables us to see the quality of the chromosome. The Tables give the biochemical parameters but do not reveal the quality of the chromosomes. Juice has been changed to extract in Figure 2.</p> <p>Line 247: HW and CW have been used successfully throughout the manuscript. Line 249-251: the mechanism has been effected.</p>
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	<p>Line 285: Change “no anti-lipid peroxidation activity” to “induce lipid peroxidation”</p> <p>Line 290-291: Which kind of compounds possesses antioxidant capacity in the extracts? Enlist some of them and refer to other studies.</p> <p>Line 312-325: Compare the obtained results with other in which the extracts of plants are tested for genotoxicity.</p>	<p>Line 252-256: statements have been modified.</p> <p>Line 259-260: phytochemical species and references are inserted.</p> <p>Line 263-266: Some of the volatile constituents might have evaporated during the hot water extraction. Extraction method is a factor that affect presence of secondary metabolites.</p> <p>Line 278-281: changes have been effected sir.</p> <p>Line 285: change has been made.</p> <p>Sir the study was not on phytochemical study or screening nor was it an identification and characterization study involving structural elucidation of a named compound. This is an investigative study involving anti-inflammatory, anti-oxidant and genotoxicity activities.</p> <p>Line 312-325: the results have been compared with other studies with references well cited. Thank you sir.</p>
<b>Minor</b> REVISION comments		
<b>Optional/General</b> comments		

**PART 2:**

	<b>Reviewer's comment</b>	<b>Author's comment</b> <i>(if agreed with reviewer, correct the manuscript and highlight that part in the manuscript. It is mandatory that authors should write his/her feedback here)</i>
Are there ethical issues in this manuscript?	<i>(If yes, Kindly please write down the ethical issues here in details)</i>	