

# Review of: "The use of Phytochemical, GC-MS Analysis and Hepatoprotective Effect of the Methanol Leaf Extract of *Camellia Sinensis* (L.) Kuntze on Paracetamol-Induced Liver Injury in Wistar Rats"

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Potential competing interests: No potential competing interests to declare.

Phytochemical Contents, GC-MS Analysis and Hepatoprotective Effect of the Methanol Leaf Extract of *Camellia sinensis* (L.) Kuntze on Paracetamol-Induced Liver Injury in Wistar Rats

Accepted with major considerations

Dear researchers, I consider that the work is interesting and has tools, equipment, and methodology that make it possible to have reliable results; however, the manuscript presents a series of errors in the experimental design as well as few details in the description, added to the fact that the images are little clear and provide little information.

On the other hand, methanolic extracts of *Camellia sinensis* with hepatoprotective activity have already been reported, which makes it difficult to understand the innovative part of the work.

Below, I describe a series of considerations that I hope will add positively to your work.

- In the title, rewrite *Camellia sinensis* (L.) for *Camellia sinensis* (L.)
- You should add a parenthesis after (3,900 ft.)
- You should add a space in "extract ofC." located in the first line of the fifth paragraph
- In section 2.2, Preparation of plant material and extraction, check if the Celsius degree symbol is correct in 25°C

I suggest reviewing the wording in section 2.2, Preparation of plant material and extraction.

- Should indicate the methanol concentration of the aqueous solution
- Indicate the difference between stirring and shaking and describe the procedure in more detail.
- Is it correct to use the room temperature of the thermostatic bath to concentrate your extract? It is generally concentrated using a rotary evaporator and at higher temperatures, since it is a sample that has water.

In section 2.3, Preliminary phytochemistry of *C. sinensis* methanol leaf extract

- You should add a space between the words "... extractusing..." in the first line of this paragraph
- I suggest indicating the citation of the procedures used for qualitative detection
- In this section, the name "cardiac glycosides" is mentioned; however, in Table 2, the reagent used to verify the

presence of this group of compounds does not appear.

In section 2.4

In section 2.2, it is indicated that an extract of leaves and stem bark is prepared; however, in section 2.4, the GC-MS analysis of the stem bark extract is carried out. I suggest that it should be clarified if these are two different extracts, one from the leaf and the other from the stem bark, or if it is a single extract prepared from both parts of the plant.

In the case of two different extracts, it should indicate why the GC-MS analysis is only performed on the stem bark extract.

Temperature units should be written with the symbol “ °C”

In section 2.5

Review the preparation of the sodium carbonate solution. It should indicate the number of grams dissolved in 10 mL of water to obtain a final concentration of 7.5%

Review the text. Below equation, mg gallic acid **equipment** should be changed to gallic acid **equivalent**

In section 2.6

Review the text. Describes an avocado seed extract which is incorrect.

Review the text. Below equation mg rutin **equipment** should be changed to mg rutin **equivalent**

Since a calibration curve will be used for the quantification of phenolic compounds and flavonoids, I do not consider it necessary to write the equations in sections 2.5 and 2.6

Section 2.8

- It should be indicated the meaning of CSE since it is the first time it appears in the manuscript

Section 2.9

- It should be indicated how the sacrifice is carried out

## **Results**

Section 3.3

- Graph 2a and b should be corrected since absorbance should be plotted as a function of the amount in micrograms of the standard and not as a function of the concentration. In these same graphs, values appear with a comma as a decimal separator, which should be changed by points both on the absorption axis and in the equation.
- In both graphs the line should pass through the intersection at 0, since it is known that it is the only value where the standard is not present.
- In graph 2 b the first three points do not present a linear relationship. This calibration curve should be repeated, and the

lower and upper detection limits taken into account.

In any case, it is not necessary to add the calibration curve graph, since it is a standard procedure and does not add any significant new data.

I consider the results corresponding to table 4 should be in a separate section.

Check when referring to the plant extract, whether it is a leaf extract, a stem extract or a mixture of both. Since in the results section it described a leaf extract, while in previous sections it described a stem bark extract.

Perhaps referring to the extract with the abbreviation CSE would be correct

With respect to table 4,

The title of table four should be written at the top of the table.

The word methanol in the title must be written without italics.

The description of the table details: ...Results are mean  $\pm$  SD of six rats per group ( $n = 6$ ),  $*p < 0.05$  statistically significant ( $P = 0.000$ ).... should be inscribed as a table footer

Some explanation should be provided as to why, at lower concentrations (200 mg/kg) of the extract, there are increases in the concentration of uric acid, and at higher concentrations, it remains unchanged.

On the other hand, the uric acid value of group V is indicated to present significant differences with the control. However, the value is the same. The statistical analysis should be checked.

In Table 5, the title should be written at the top of the table.

Clarifications on statistical treatment and abbreviations should be written as a table footer.

Regarding the results obtained in terms of protein concentration (TPRN), according to the statistical analysis, there are no significant differences with group II, whose values are higher than the control, but there are differences with group III, whose value is closer to the control. I think you should review the statistical analysis.

It should be clarified whether the analysis is carried out with respect to the normal control or the disease control.

Figure 3 is very unclear; it is very pixelated, and the circles are also imprecise. I suggest changing the images to sharper ones. Replace the circles with arrows. Furthermore, I consider that since it is a histological section, the central vein, sinusoidal lining, hepatic artery, hepatic vein, hepatocytes, and pyknotic nucleus should be indicated.

## Discussion

In the second paragraph in the discussion section, it is cited that "Camellia sinensis has been reported to be a good antioxidant, anticancer, and antiinflammatory medicinal due to the presence of these metabolites (Zhao et al., 2022), which corroborated the findings from this present study". However, in this work, it did not have any experience that would

allow one to assert that this excerpt possesses these activities.

On the other hand, its GC-MS spectrum demonstrates a composition rich in fatty acids and fatty acid derivatives. However, it does not discuss whether there is a report in the literature of hepatoprotective activity of the fatty acids detected in its analysis.

I consider that you should highlight the phenolic compounds and flavonoids that you detected in your extract that have already been reported with hepatoprotective activity.

In its results, at the dose of 400 mg/kg, there was an increase in globins similar to the effect of paracetamol. Wouldn't this be counterproductive?

I consider that the discussion should be expanded by justifying the selection of the selected biomarkers.

Regarding the discussion of the histological study, again, I think the images are unclear.

### **Major considerations**

An analysis of the effect of administering the extract alone, that is, without administering paracetamol, should have been carried out to verify whether the extract has a toxic effect.