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OPEN PEER REVIEW

Characteristics and Hepatoprotective Activity of the *Curcuma heyneana* Rhizome Extract toward Wistar Rats Induced by Ethanol

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About this report

This open peer review (OPR) report shows the public comments made on this article during peer review, by the reviewers, the associate editor and the editor-in-chief, for every revision round, together with the authors' replies. Comments appear only with the consent of the people who wrote them. Reviewers who chose to sign their report are named; the others are shown as "Referee". Comments are edited for language only, and confidential remarks to the editors are not included.

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1 Original submission

3 comments · 10 Jan 2021 – 31 Jan 2021

Mahmoud Omidi Reviewer

ACCEPT 10 Jan 2021

Dear AE;

This is an excellent report of very thorough research. The literature review was thorough, and the methodology was thorough. The sample size was enough. The present research has sufficient novelty in the related area.

Best regards!

Referee 1 Reviewer

MAJOR REVISION 10 Jan 2021

Dear AE;

Comments for the author:
1) Abbreviations should be written in full in the abstract.
2) The names of liver enzymes should not be written in italics in the introduction.
3) Please cite these 2 articles in this manuscript.
A) Protective effect of elaeagnus angustifolia L. Fruit hydroalcoholic extract on cyclophosphamide-induced nephrotoxicity in mice.
B) Protective effect of hydroalcoholic extract of lavandula officinalis L. on gentamicin induced nephrotoxicity in rats.

Best!

Referee 2 Reviewer

REJECT 31 Jan 2021

Dear AE;

comments for authors;
The scientific name of the plant is not complete.
Some results are missing from the abstract.
The text should be revised grammatically.
The time of sample collection should be added.
The result of *Total curcuminoi*d should be moved to the results section.
The procedure of extraction is not proper. This extract can be full of non-volatile compounds. The direct injection of this extract into GC-mass is incorrect. So the results indicated in table 2 may be invalid.
The discussion is very concise; this section must be extended, and the results must be explained based on compounds isolated from this plant in previous studies.

Best regatds!

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Revision 1

AUTHORS' RESPONSE TO REVIEWERS

Authors' reply to reviewers

The next pages contain the authors' point-by-point reply to the comments of this revision round, as submitted with the revised manuscript. It is published as the authors wrote it.

ANSWER TO REVIEWER COMMENTS

REVIEWER 1 COMMENTS	
COMMENT DETAILS	**AUTHOR ANSWER**
This was an excellent report on very thorough research. The literature review was thorough, the methodology was painstakingly thorough and incorporated the use of sufficient numbers of samples in size analysis and tests. Morever, the present research has sufficient novelty in the related area.	Thank you for the comment
REVIEWER 2 COMMENTS	
COMMENT DETAILS	**AUTHOR ANSWER**
1) Abbreviations should be written full in abstract.	I have written full sentence before abbreviations toward GC-MS, AST, ALT, ANOVA, GAE, QE
2) The names of liver enzymes should not be written in italics in the introduction.	I have revised the written of alanine transaminase and aspartate transaminase

3) Please be cited 2 article in this manuscript. A) Protective effect of elaeagnus angustifolia L. Fruit hydroalcoholic extract on cyclophosphamide-induced nephrotoxicity in mice. B) Protective effect of hydroalcoholic extract of lavandula officinalis L. on gentamicin induced nephrotoxicity in rats.	The article metioned by reviewer 2 are not suitable to cite for our study, we have conducted the study about hepatoprotective activity, NOT nephrotoxicity.
REVIEWER 3 COMMENTS	
COMMENT DETAILS	**AUTHOR ANSWER**
The scientific name of plant is not complete.	The complete name of plant from Herbarium Medanense is *Curcuma heyneana* (Valeton & Zijp.). I have mentioned the full name in the initial of abstract and in Materials and Methods in plant part. I didn't put the full name of plant in the whole of paper, because of the length of the name. Therefore I shortened to be *C. heyneana*.

Some results were missed in abstract.	I have add the result of total curcuminoid in abstract that is in methods and in results part.
The time of sample collection should be added to plant part.	I have add the time of rhizome collection or harvesting
The result of $Total\ curcuminoi$d should transfer to result part.	I have add the result of total curcuminoid in the abstract and the result part
1. The procedure of extraction is not proper. 2. This extract can be full of non-volatile compounds. The direct injection of this extract to GC-mass is incorrect. So the results was indicated in table 2 may be invalid. 3. The discussion is so concise, this section must be extended and explain the result based on isolated compounds of this plant in previous researches.	1. I have revised the extraction procedure based on Indonesian Pharmacopoeia. 2. The thick extract of *C. heyneana* was diluted with ethanol before injected to the GC-MS. Some compounds that was detected in mass spectra mostly the same as compounds detected in another plant like *Zingiber officinale* (bisabolene), *Artemisia vulgaris* (eucalyptol), *Andrographis panniculata* (andrographolid) and *Coriandrum sativum* (linalool) as I described in discussion part. Therefore, I hope the data presented in Table 2 is valid. 3. I have add explanation in the discussion and make the correlation between

	chemical compound in *C. heyneana* and previous research
EDITOR IN CHIEF COMMENTS	
L57, add the country of Merck company.	I have add the country of Merck company
- L78, explain all the parameters in the mentioned equation. - What is 10000 in the equation mentioned here? - Add an equation number and refer to the number instead of saying “equation below.” The same for all equations used throughout the manuscript e.g. L98, 109, 126 and .....; Eq.1 , Eq.2, and so forth	- Total curcuminoid means amount of curcuminoid contained in the extract of *Curcuma heyneana* rhizome. Concentration of curcuminoid means the data of curcuminoid content in the sample/extract that is gained from regression equation of standard curcuminoid. Regression equation is y=0.158372 x + 0.0191192, correlation coefficient r2 = 0.99896 - The absorbance initially conducted toward pure compound of curcuminoid. After getting standard curve, amount of curcuminoid in extract was carried out using Eq. 1. Last addition volume means amount of solvent added in the sample that is 10,0 ml. Dilution factor is amount of dilution. Sample weight is the amount of sample used. In this research, I can show the data of calculation of total curcuminoid.

	Sample weight: 1.063 g Last addition volume: 10.0 ml Dilution factor: 25 Absorbance result: 5.003 ppm Total curcuminoid: 1.18% - I have revised the Eq. 1 - I have revised as your advice. I have add the equation number and refer to the number instead of saying equation below.
L81, add the detail of GC/MS equipment, kind of MS detector, software, and temperature programming, as well as injected volume. How do you inject the sample; I mean, the kind of solvent and how it is diluted?	- GC/MS equipment: Gas chromatography (Thermo Scientific™ TRACE™ 1310 Gas Chromatograph, United States of America) – mass spectrometry (Thermo Scientific™ ISQ™ LT Single Quadrupole Mass Spectrometer, United States of America) - MS detector: flame ionization detector (FID) and electron capture detector (ECD) - Software: Thermo Scientific™

	Chromeleon™ 7.2. - Column: HP-5MS UI 1. Length: 30 m 2. I.D.: 0.25 mm 3. Film: 0.25 µm 4. Max Temp: 325/350°C - Instrument condition: 1. Gas carrier: Helium UHP (He) 2. Injector temperature: 260 °C 3. Split flow: 50 ml/min 4. Split ratio: 50 5. Front inlet flow: 1,00 ml/min 6. MS transfer line temp: 250°C 7. Ion source temp: 200°C 8. Purge flow: 3 ml/min 9. Gas saver flow: 5 ml/min 10. Gas saver time: 5 min

	- The solvent of extract is ethanol, because the extraction process of the rhizome of *C. heyneana* carried out using ethanol. Therefore dissolving the extract with ethanol may dissolve all chemical substance in the extract. - I have add all explanation in the article
Which MS library or libraries do you use to identify the compounds?	Interpretation on mass-spectrum GC-MS was conducted using the database of National Institute Standard and Technology (NIST) 2014
How do you check the similarity? Did you calculate the Kovat index for the compounds? Ithe similarity supposed to be mentioned in percent; what do the numbers under similarity mean?	- Q: How do you check the similarity? A: Similarity was conducted based on retention time and m/z. Identification of volatile compounds was carried out by comparing MS spectrum and RIs (retention indices (RIs) of components in ECG with those of the authentic standards in NIST library (2014).

	- Q: Did you calculate the Kovat index for the compounds? A: We didn’t calculate Kovat index for the compounds? - Q: what do the numbers under similarity mean? A: In the comparison of MS spectrum, the requisites should be that both SI and RSI were more than 800. Therefore, from the result of MS spectrum, we took the compound that has SI and RSI more than 800. Not only that, we also took the abundant compound (the highest peak) even though SI and RSI below 800
Add area percent reported in library search of GC/MS to table 2. Use the area normalization method to show the extract's	We have no data about area percent of the compound. However, I have revised Table 2.

relative concentration, especially the active compound you have mentioned here.	If the data of GC MS is not fulfilling your criteria, I propose to withdraw this data from this article.
Did you check the peak purities?; especially for curcumenol?	I didn’t check the peak purities for curcumenol
Remove peak number in GC/MS total ion chromatogram; present a more clear chromatogram.	The instrument released the chromatogram with number. The number indicate the chemical constituent in the extract. There are 86 peaks. Every peak in the MS spectrum was matched with NIST data base to determine kind of chemical substance.
Standard error in Table 3 and 4	I have add the standard error in Table 3 and Table 4

2 Revision 1

3 comments · 7 Mar 2021 – 6 Apr 2021

Referee 1 Reviewer

ACCEPT 7 Mar 2021

Dear AE;

In this form the manuscript is accepted.

Best!

Referee 3 Reviewer

MINOR REVISION 6 Apr 2021

Dear AE;

comments for the author:
Thank you for all the corrections you made and also for the logical answers in most cases.
Only one point still needs to be corrected. It seems that reviewer 3 means the results of microscopic and macroscopic examinations when referring to "missing data in abstract", as the results for curcuminoid were already included in the abstract. Please correct this item too.

Best regards!

Editor-in-Chief

MINOR REVISION 6 Apr 2021

Dear Authors,

Your revised manuscript was scrutinized, and it seems that some points should be considered before a final decision is made. The reviewer's comments are included, and the following points should be addressed as well.
L71: Add the exact amount of C. heyneana rhizome powder that was used.
- The authors have provided all the details of GC-MS, but these have not been fully added to the revised manuscript; add the details to make it easy for the reader to reproduce your work, e.g., the dilution factor for the injected sample and whether the injection method was split or splitless. Include details on how you determined SI/RSI in Table 2. Define SI and RSI in the revised manuscript. You have discussed similarities without explaining how they were calculated.

- For GC-MS instrumentation and conditions, see how reference “J. Agric. Food Chem. 2002, 50, 3789–3794” has presented the instrument information. In your answer, you have considered FID and ECD! Have you used them? It makes no sense; your GC detection systems are supposed to be MS!
- The area percentage of the compound is written exactly beside the compound's retention time in the GC-MS report; it would be better to add it to Table 2.
It makes no sense to have a peak number on every peak in the GC-MS total chromatogram; see reference “J. Agric. Food Chem. 2002, 50, 3789–3794”; one can use GC-MS processing software to remove the peak numbers. If this is difficult for you, I suggest removing the chromatogram; the peak table (Table 2) works well.
- Since the authors are testing the protective effect of the raw material, I suggest adding a few sentences to the revised manuscript (conclusion section) describing this and suggesting possible future research on extract

the raw extract using multiple techniques? Explain in terms of the interactions of the assayed compounds and their possible protective effect.
- Check the order of the uploaded figures! Fig. 4 appears before Fig1!
- Last but not least, the manuscript needs English polishing. In the Abstract: “Extract at the doses of 50, 100, and 14 150 mg/kg inhibited the elevation of AST and ALT which is significantly different from the 15 negative control group ($p<0.05$)” would be better phrased as “Extract at the doses of 50, 100, and 14 150 mg/kg inhibited AST and ALT elevation, which is significantly different from the 15 negative control group ($p<0.05$). ” Another example is “Among 152 them peak number 2, 3, 5, 6, 8, 9, 14, 27 and 34 are them which have”: “them” and “them”! Check for other typos as well.

We are looking forward to receiving your revised manuscript as soon as possible.

Kind regards,

Zahra Ramezani
JJNPPEditor-in-Chief

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Revision 2

AUTHORS' RESPONSE TO REVIEWERS

Authors' reply to reviewers

The next pages contain the authors' point-by-point reply to the comments of this revision round, as submitted with the revised manuscript. It is published as the authors wrote it.

ANSWER TO REVIEWER COMMENTS

REVIEWER 1 COMMENTS	
COMMENT DETAILS	**AUTHOR ANSWER**
Accept	Thank you for your kindness
REVIEWER 2 COMMENTS	
COMMENT DETAILS	**AUTHOR ANSWER**
Thank you for all corrections you made and also for the logic answers in most cases. Only one point still should be corrected. It seems the reviewer 3 means the results of microscopic and macroscopic examinations with the "missing data in abstract". As the results of curcuminoid had already existed in the abstract. Please correct this item too. Good Luck!	Thank you, I have revised the abstract as follow: Nine compounds were identified in the extract. Administration of extract at the doses of 50, 100, and 150 mg/kg keep the liver normal. It was identified macroscopically from the dark red colour without any white spot and normal liver weight. Furthermore, extract at the doses of 50, 100, and 150 mg/kg inhibited AST and ALT elevation, which was significantly different from the negative control group ($p<0.05$). The extract also prevented

	hepatocytes injury that was seen microscopically.
REVIEWER 3 COMMENTS	
COMMENT DETAILS	**AUTHOR ANSWER**
Some results were missed in abstract.	I have add the result of total curcuminoid in abstract that is in methods and in results part. I also add amount of chemical compounds identified with GC-MS as well as macroscopic and microscopic result as follow: Nine compounds were identified in the extract. Administration of extract at the doses of 50, 100, and 150 mg/kg keep the liver normal. It was identified macroscopically from the dark red colour without any white spot and normal liver weight. Furthermore, extract at the doses of 50, 100, and 150 mg/kg inhibited AST and ALT elevation, which was significantly different from the negative control group ($p<0.05$). The extract also prevented hepatocytes injury that was seen

	microscopically.
The time of sample collection should be added to plant part.	I have add the time of rhizome collection or harvesting as follow: The rhizome was taken from PT. Sumatera Busan, Medan, Indonesia that was harvested in July 2018.
The result of $Total\ curcuminoi$d should transfer to result part.	I have add the result of total curcuminoid in the abstract and the result part
1. The procedure of extraction is not proper. 2. This extract can be full of non-volatile compounds. The direct injection of this extract to GC-mass is incorrect. So the results was indicated in table 2 may be invalid. 3. The discussion is so concise, this section must be extended and explain the result based on isolated compounds of this plant in previous researches.	1. I have revised the extraction procedure based on Indonesian Pharmacopoeia. 2. I have repeated to detect the compound in *C. heyneana* using GC-MS. I presented the new chromatogram in Figure 1 in the result part. Besides that, I also have revised the content of Table 2, based on the latest result and your suggestion to follow the article in the Journal of Agricultural and Food Chemistry. I also revised the procedure about “Determination of chemical substances using gas chromatography-mass spectra (GC-MS)” in the Materials and Methods Part. 3. I have add explanation in the discussion

	and make the correlation between chemical compound in *C. heyneana* and previous research
EDITOR IN CHIEF COMMENTS	
L71: add the exact amount of the rhizomes of C. heyneana powder that has been used.	In the determination of curcuminoid, 0.1063 g extract was used
The authors have provided all the details of GC-MS, but they are not fully added in the revised manuscript; add the details to make it easy for the reader to reproduce your work. e.g dilution factor for injected sample; the method of injection was split or split-less; Include details for how did you determine SI/RSI in Table 2? Define SI and RSI in the revised manuscript. You have talked about similarities without explaining how they have been obtained calculated.	I have provided the details of GC-MS, dilution factor, method of injection. I didn't determine SI/RSI since I change the data of GC-MS and the way I presented the data in Table 2. The compound was determined by matching with the compound in the library and I have written it in the method.
- for GC/MS instrumentation and conditions, see how reference "J. Agric. Food Chem. 2002, 50, 3789−3794" has presented the instrument information. In your answer, you have thought of FID and ECD!! have you used them? It makes no sense; your GC detection systems are supposed to be MS!	- Thank you for your recommendation. I have revised the GC-MS result based on the article in the J. Agric. Food Chem. 2002, 50, 3789−3794 journal. - The detector of GC that we used is MS, not FID or ECD, I am sorry for the

	mistakes.
- Area percent of the compound is written exactly beside the compound retention time in the GC_MS report; It is better to be added to Table 2. It makes no sense to have peak number on all peaks about GC-MS total chromatogram; see reference “J. Agric. Food Chem. 2002, 50, 3789−3794”; one can use GC-MS processing software to remove it; if it is difficult for you, I suggest removing the chromatogram; peak table (Table 2) works well.	- Thank you for your advice. I have rechecked the compound in the extract using GC-MS. I presented new chromatogram in the result part and tried to follow the journal. - I have put area percent of the compound in the Table.
- Since the authors are testing the protective effect of raw material, I suggest adding a few sentences in the revised manuscript (conclusion section) describing this and offering the probable future research on the extract fractionation and finding the exact effective constituent.	I have revised the conclusion as follow: It can be concluded, the extract of *C. heyneana* at the doses of 50, 100 and 150 mg/kg effective as hepatoprotective in ethanol caused the liver injury. Further research is needed to do extract fractionation in order to get pure compound which is responsible for the hepatoprotective effect.
- in discusion: Make it straightforward for users; why have you determined different constituents of the raw extract by multiple techniques; explain from an interaction point of view of the assayed compounds and their possible protective effect.	I have revised

- Check the order of Figures uploads! Fig. 4 before Fig1!!	I will arrange the figure as in order number
- Last but not least, the manuscript needs English polishing. In Abstract: “Extract at the doses of 50, 100, and 14 150 mg/kg inhibited the elevation of AST and ALT which is significantly different from the 15 negative control group ($p<0.05$)” it is better to say “Extract at the doses of 50, 100, and 14 150 mg/kg inhibited AST and ALT elevation, which is significantly different from the 15 negative control group ($p<0.05$). “ or “Among 152 them peak number 2, 3, 5, 6, 8, 9, 14, 27 and 34 are them which have” them and them!!; and check for the other typos.	Thank you, I have revised as your advice and I have highlighted the sentences.

3 Revision 2

1 comment · 25 May 2021

Editor-in-Chief

ACCEPT 25 May 2021

Dear Authors,

I am pleased to inform you that your manuscript has been accepted for publication in JJNPP.

Thank you for considering JJNPP; we are looking forward to receiving your future research results.

Kind Regards,
JJNPP EIC