Author’s response to reviews

Title: Thai plants with high antioxidant levels, free radical scavenging activity, anti-tyrosinase and anti-collagenase activity

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Author’s response to reviews:

Answer to Reviewer 1

We thank the reviewer for the constructive comments on our manuscript. We will detail in our response below how we plan to address the reviewer comments.

* Some statements are not clear for example, Page 9, Line 186-187: "This is double…"

Answer We changed that sentence.

* For some plants the said activities were reported and the part of the plant possessing the highest amount of phytochemical content was different that used in the present study (for example for A. squamosa roots and bark than leaf). The authors should explain why they selected the leaves than other parts of the plants.

Answer: We used only leaves of these plants because in Thai traditional medicine, only leaf part was used to treat skin disease, but the mechanism is not clear, so, we would like to examine their properties.

* The authors used three solvents but it is not very clear why they chose these solvents. The best choice would have been dichloromethane, ethanol and water.

Answer: We would like to extract the plants with different solvents to find different active compounds. We used Soxhlet apparatus extraction which is not suitable for water extraction, so for high polar solvent, we used only ethanol (to mimic traditional medicine preparation process). We used petroleum ether, dichloromethane and ethanol to cover all range of high, medium and
low polarity active compound. Moreover, petroleum ether can extract non-polar compound from plants which may contain anti-tyrosinase and anti-collagenase compound.

* What was the basis for doing correlation analysis of total phenolic and flavonoid content? Flavonoid content was not correlated but total phenolic content was correlated with antioxidant activity. What is the meaning of this finding?

Answer: We would like to know correlation analysis between phenolics and flavonoids. If the plant has more phenolics, it may have more flavonoids. The results showed that phenolics were not correlated with flavonoids. We removed this correlation.

* Is there any relationship between phytochemical content, antioxidant activity and enzyme inhibition activity? I raised this issue because ethanol extract of Ardisia elliptica Thunb., Annona squamosa L. and Senna alata (L.) Roxb. inhibited both tyrosinase and collagenase. However, Rhinacanthus nasutus, which has low phytochemical contents and antioxidant activity, surprisingly exhibited the highest tyrosinase inhibiting activity but no collagenase activity.

Answer: It has not totally relationship between phytochemical content, antioxidant activity and enzyme inhibition activity. In next project, we may analyze the individual active compounds from some potential plants and test those pure compound for the properties.

* The authors said that they used student t-test for analysis. But, I did not see anything about the analysis in the text.

Answer: I used student t-test analysis for anti-tyrosinase and anti-collagenase activity. I wrote under the table of results that we compared the sample with control group as 100% (not treat with plant extracts).

Answer to Reviewer 2

We thank the reviewer for the constructive comments on our manuscript. We will detail in our response below how we plan to address the reviewer comments.
1. The major concern is regarding to the antioxidants properties from DMSO itself. DMSO has been documented able to reduce lipid peroxidation, protein carbonyl formation, hydroxyl radical production. Authors used DMSO as solvent in this study to determine the antioxidative function and therefore need to clarify the potential contribution by DMSO itself.

Answer In this issue, we tested DMSO which showed phenolic content about 1 mgGAE/g dry weight, flavonoid content about 0.8 mgQE/g dry weight, DPPH and ABTS (not detected scavenging activity). We used DMSO as a control (not treated with plant extracts).

2. The extracts from plant by either ethanol or other solvent will produce just a raw mixture of various compounds. The inhibitory effect of either tyrosinase or collagenase might be due to combination of various compounds, so does antioxidants properties. In addition, the toxicity of the extract might over balance the antioxidants properties in these extracts. Authors need to clarify the limitation of their study.

Answer This study is to screen some activities of plant extracts. We will test toxicity of the extracts and separate active compounds in next projects. Concentration of this study cannot use directly in human. And I will clarify in conclusion section.

3. In phenolic, flavonoid content test, a DMSO blank data is needed.

Answer We has already tested DMSO blank.

4. In line 186, don't understand the sentence "This is double your results which is more than the content in a twig extract"

Answer I’m sorry. We forgot to remove this sentence. I edited this sentence.

5. English proofreading is needed in entire text.

Answer We’ve proofread our text already.

‘Answer to Reviewer 3

We thank the reviewer for the constructive comments on our manuscript. We will detail in our response below how we plan to address the reviewer comments.
- Many plants tested in the present study (Senna alata, Annona squamosa, Garcinia mangostana, Ardisia elliptica...) have been already screened for their antioxidant properties. Thus, this objective seems to be not very important.

Answer Those plant may be tested for anti-oxidant activity, but if we used different extraction methods or solvents, the outcome may differ from the previous one. We would like to examine antioxidant property from plant extract, using different solvents or meth...

- Also, one of the objective was to test the anti-wrinkle effects of the selected plant extracts. In my opinion, I think this objective has been partially completed since 4 plants out of the 14 used in the study have been screened for this purpose (see results on collagenase inhibition test)

Answer: We select only high potential plants (highest antioxidant property) for studied about anti-wrinkle effect.

- The authors should provide IC5O values which give a better representation of the potency of a drug

Answer We provided IC50 for some plants which showed activity more than 50% (line 275 and 299).

- in the abstract section, results with Cassia alata are mentioned but this plant is missing on the list of plant tested the authors provided

Answer Results of Senna alata have already showed in tables of antioxidant properties, tyrosinase and collagenase activity.

- Concerning the background section, I suggest the authors remove the paragraph starting from line 68 to 73 because it is a summary of the methodology, results and conclusion sections.

Answer We removed those lines.

- The authors should provide results with reference drugs

Answer We used kojic acid as reference drug for tyrosinase (line 270) and epigallatecatechin gallate for collagenase (line 297). We put these results in the table.