Author’s response to reviews

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

Authors:
Anchalee Chiabchalard (anchalee.c@chula.ac.th)
Moragot Chatatikun (moragotc@gmail.com)

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Dr. Seung Min Oh (Associate Editor),
BMC Complementary and Alternative Medicine

Dear Dr. Seung,

Thank you for giving the opportunity to revise and resubmit our manuscript, Thai plants with high antioxidant levels, free radical scavenging activity, anti-tyrosinase and anti-collagenase activity, BCAM-D-17-00645R1. We appreciate the time and detailed analysis provided by each reviewer and by you. This manuscript has certainly benefited from the insightful suggestions.

I have responded specifically to each suggestion as detailed below. Please find the changes highlighted in yellow and a point-by-point response to the reviewers’ comments.

We look forward to seeing our manuscript in BMC Complementary and Alternative Medicine.

Sincerely,
Anchalee Chiabchalard, Ph.D.
Reviewer 1
Ephrem Engidawork, PhD

1. Authors addressed by and large my concerns, except that the aqueous extract could have still been obtained by boiling with water the residue following extraction by the organic solvents.

Response: Thank you for your suggestion. In this study, we used soxhlet apparatus for extraction which is not suitable for water. We screened only three extracts namely petroleum ether, dichloromethane and ethanol which covered almost all polarity of the active ingredients. We found that the ethanol extracts have anti-tyrosinase and anti-collagenase activity. Hence, water extracts were not used in this study. In the next project, I will keep your suggestion to use water extracts. Your suggestion is very useful for us about extraction.

2. The asterix shown in Table 5 and 6 need to be explained as a footnote below the Table. For example, it says significantly different from the control. Does control refer to the two standards used? If so, I do not expect a difference between the ethanol extract of Ardisia elliptica Thunb. and the standard chemical used.

Response: We calculated tyrosinase inhibition from $100 \times \frac{(A492 \text{ of control} - A492 \text{ of sample})}{A492 \text{ of control}}$. We compared sample test and positive control with control (without sample).

Control well = enzyme buffer

Sample well = plant extracts at 1 mg/ml with enzyme buffer

Kojic acid well (positive control for tyrrosinase inhibition) = kojic acid at 0.1 mg/ml with enzyme buffer

Epigallocatechin gallate (positive control for collagenase inhibition) = epigallocatechin gallate at 0.1 mg/ml with enzyme buffer

So, kojic acid has greater tyrosinase inhibition than Ardisia elliptica Thunb. Because we used kojic acid at 0.1 mg/ml which is less than Ardisia elliptica Thunb (1 mg/ml). As same as, we used epigallocatechin gallate at 0.1 mg/ml which is lower concentration than Ardisia elliptica Thunb.
3. The language still needs revisiting.

Response: Our manuscript has been reviewed for English language usage by Professor Dr. Duncan R. Smith, Mahidol University, Thailand.

Reviewer 3
Nguelefack-Mbuyo Pami Elvine, Ph.D

1. The authors provide satisfactory answers to the points I raised. But I would like to know how they succeed in calculating IC50 values using just one concentration of plants extracts during their assays. The way the IC50 values were obtained should be explained in the manuscript.

Response: Thank you for your comment. We didn’t use only one concentration. If some extracts show the ability more than 50%, each extract was diluted into two fold dilution. We plotted a graph of percent inhibition against concentration at 0.78, 1.56, 3.125, 6.25, 12.5, 25, 50, 100 µg/ml of each extract for DPPH and ABTS assay and at 15.62, 31.25, 62.50, 1250 250, 500, 1000 µg/ml of each extract for tyrosinase and collagenase activity. I put the determination of IC50 in the method section, line 124-125, page 5; line 138-139, page 6; line 149-150, page 7; line 162-163, page 7.