Author's response to reviews

Title: Assessment of phytochemical content, polyphenolic composition, antioxidant and antibacterial activities of Leguminosae medicinal plants in Peninsular Malaysia

Authors:

Yik Ling Chew (yikling82@yahoo.com)
Wan Ling Elaine Chan (elainechan87@yahoo.com)
Pei Ling Tan (ashleytan23@gmail.com)
Joo Kheng Goh (goh.joo.kheng@sci.monash.edu.my)
Yau Yan Lim (Lim.Yau.Yan@sci.monash.edu.my)
Johnson Stanlas (rcxjs@medic.upm.edu.my)

Version: 2 Date: 6 December 2010

Author's response to reviews: see over
Responses to reviewers’ comments.

Title: Assessment of phytochemical content, polyphenolic composition, antioxidant and antibacterial activities of Leguminosae medicinal plants in Peninsular Malaysia

Authors:
Yik-Ling Chew: yikling82@yahoo.com
Elaine-Wan-Ling Chan: elainechan87@yahoo.com
Pei-Ling Tan: ashleytan23@gmail.com
Yau-Yan Lim: Lim.Yau.Yan@sci.monash.edu.my
Johnson Stanslas: rcxjs@medic.upm.edu.my; jstanslas@yahoo.co.uk
Joo-Kheng Goh: goh.joo.kheng@sci.monash.edu.my

Author’s response to reviews: Please see over.
Dear Editor

Please find below the responses made to the reviewers’ comments. The revised manuscript is attached for your consideration for publication. This manuscript has not been submitted simultaneously for publication elsewhere. We hope the revised manuscript is acceptable.

Yours sincerely,

Dr. Goh JK

Responses to Reviewers’ comments:

Reviewer 1: Ilham Gülçin

Reviewer's report:

In the present study the authors evaluated the phytochemical content, polyphenolic composition, antioxidant and antibacterial activities of Leguminosae medicinal plants such as Acacia auriculiformis, Bauhinia kockiana, Bauhinia purpurea, Caesalpinia pulcherrima, Calliandra teregmina, Cassia surattensis, Leucaena leucocephala, Peltophorum pterocarpum, and Samanea saman in Peninsular Malaysia. The manuscript was well-organized and clear and concise.

After some corrections, it can be accepted as regular manuscript. These corrections are listed below:

1. Page 2/In Results: Some results related to antioxidant activity should be given in related section of abstract.

Response: Results on the antioxidant activity are given (p.3).

2. Page 4: After the paragraph ‘One of the major groups of phytochemicals, phenolic compounds, …. bilayers against peroxidation induced by reactive oxygen species (ROS) [6].’ The both following related reference should be given: ‘Polyphenol contents and antioxidant activity of lyophilized aqueous extract of propolis from Erzurum, Turkey. Food and Chemical Toxicology, 48, 2227–2238’ and ‘Antioxidant properties of resveratrol: A structure-activity insight. Innovative Food Science and Emerging Technologies 11, 210-218’.

Response: References suggested have been included included (p.5).

3. Page 34: The authors should be deleted unnecessary Table 4 from the text.

Response: Unnecessary Table 4 in the text has been deleted (p.18 and p.19).

Level of interest: An article of outstanding merit and interest in its field

Quality of written English: Acceptable
Statistical review: No, the manuscript does not need to be seen by a statistician.

Reviewer 2: Victor Kuete

Reviewer's report:
The work reported Yik-Ling Chew et al. provide a baseline information for future pharmacological work on nine Malaysian medicinal plants. The methods are well described. However, minor revision is required to have a final acceptable form of the manuscript. below are the point to check.

1. Go through the manuscript to correct careless mistakes (Grammar, microbial strains names, e.g. pneumoniae not pneumonia.....etc ).
   Response: Manuscript is thoroughly checked. Spelling mistake ‘pneumonia’ is changed.

2. Check the IC50 value of ascorbic acid. Is it 0.00382 or 0.0382 mg/mL (The known values are closer to this one).
   Response: IC_{50} value of ascorbic acid used in our study is 0.00382 mg/mL (p.8).

3. Give IC50 values in µg/ml and refer to cutoff point for significance in discussion section (see Omisore et al. 2005; Kuete and efferth, 2010). Omisore et al. (2005) considered the cut-off point for antioxidant activity as 50 µg/ml. Samples with IC50 > 50 µg/ml were classified as being moderately active, while samples with IC50 < 50 µg/ml were judged as having high antioxidant capacity. According to Kuete and Efferth (2010), plant extracts are considered to have high or significant antioxidant capacity when IC50 < 50 µg/ml, moderate antioxidant capacity with 50 < IC50 < 100 µg/ml (extract) or low antioxidant capacity with IC50 > 100 µg/ml


Level of interest: An article of importance in its field

Quality of written English: Needs some language corrections before being published

Statistical review: Yes, but I do not feel adequately qualified to assess the statistics.

Reviewer 3: Shela Gorinstein

Reviewer's report:

1. It is written (P 2):"High total phenolic content (TPC) and strong DPPH radical scavenging ability were observed for B. kockiana flowers and C. pulcherrima leaves”.
   Comment. Please, put in data.
   Response: Results on the antioxidant activity are given (p.3).

2. It is written (P 2): ”This paper presented some of these medicinal plants are potential source of antioxidant and antibacterial agents, especially in MRSA infections treatment”.
   Comment. Please, name the “some of these medicinal plants.
   Response: The potential medicinal plants are stated, namely B. kockiana, C. pulcherrima, C. tergemina and P. pterocarpum. (p.4)

Background

3. It is written (P 3): "Natural products and secondary metabolites that are formed by living systems, notably from plant origin have shown great potential in treating human diseases such as cancer, coronary heart diseases, diabetes and infectious diseases”.
   Comment. Please, put in references.
   Response: References are included (p.4).

4. It is written (P 3): " The compounds from plants that are useful in the development of new drugs are phytochemicals. Phytochemicals are natural, non-nutritive, bioactive compounds produced by plants that act as protective agents against external stress and pathogenic attack”.
Comment. Please, put in references.

**Response:** References are included (p.5).

5. It is written (P4): "Some of these plants are medicinal plants, which have been used traditionally to treat various diseases".

Comment. Please, name the "some of these medicinal plants.

**Response:** Examples of medicinal plants are stated. (p.5)

6. It is written (P5): "... (kan cao) root extracts have chemopreventive properties where they were found to induce apoptosis in MCF-7 breast cancer cells [9]

Comment. Please, put in the kind of the extract.

**Response:** The type of extracts are given. Ethanolic extracts. (p.6)

**Materials and Methods**

7. It is written (P 9): "Phytochemical screening for flavonoids, tannins, saponins and alkaloids was determined as described by Parekh et al. [17] and Aiyegoro et al. [18] with a slight modification".

a. Comment. Please, describe the modification.

**Response:** The modification is stated. (p.10)

b. Comment. I didn’t find a description of the statistical evaluation.

**Response:** A paragraph of description of statistical analysis is given. (p.11)

**Results**

8. It is written: (P10): "Slightly different ranking was seen in TPC of the leaves: C. pulcherrima has the highest TPC (5030 ± 602 mg GAE/100g), followed by P. pterocarpum (4880 ± 275 mg GAE/100g), B. kockiana (4220 ± 104 mg GAE/100g), C.tergemina (5030 ± 602 mg GAE/100g), C. surattensis (3330 ± 309 mg GAE/100g), A.auriculiformis (2280 ± 294 mg GAE/100g), L. leucocephala (1700 ± 277 mg/GAE/100g), S.saman (1340 ± 22 mg GAE/100g) and B. purpurea (1310 ± 124 mg GAE/100g)".

Comment. Please, indicate, if the differences were significant.

**Response:** Plants which showed no significant difference are stated in the text. (p.12)

9. It is written: (P11): "Statistical analysis showed that TPC and antioxidant activity of flowers and leaves of the same species are significantly different to each other”.

Comment. Please, show data.
Response: Superscript (a-b) is stated in Table 1, where same letters (a-b) are statistically insignificant (stated below Table 1). (p.31)

Discussion

10. It is written: (P13): "Plants produce diverse arrays of phytochemicals which are useful in the development of new drugs. These phytochemicals are mostly secondary metabolites constantly synthesized by the plant for defensive purposes. For instance, antioxidants are biologically produced as defensive mechanism to prevent tissues destruction caused by highly reactive chemical species, which are formed from various biochemical reactions”.

Comment. Put in references.

Response: Reference is stated. (p.14)

11. It is written: (P15): "Our previous study [13] has shown that flavonoid is present in C. surattensis flowers and leaves extracts (13.3 mg rutin equivalent/100g and 29.6 mg rutin equivalent/100g, respectively), assessed using aluminium chloride method. Rutin (95.7 mg/100g) and chlorogenic acid (9.13 mg/100g) in extract would exhibit antioxidant activities [13]”.

Comment. Please change to "Our previous study [13] has shown that flavonoids are present in C. surattensis flowers and leaves extracts (13.3 mg rutin equivalent/100g and 29.6 mg rutin equivalent/100g, respectively), assessed using aluminium chloride method. Rutin (95.7 mg/100g) and chlorogenic acid (9.13 mg/100g) in extract would exhibit antioxidant activities [13]”.

Response: To be more specific, we have changed the word “flavonoid” to “rutin” in the sentence. (p.16)

12. It is written: (P18): "This is the first antioxidant, antibacterial and phytochemical screening study for S. saman flowers. The presence of low tannin and flavonoid content in flower (Table 4) could have contributed to medium low TPC and free radical scavenging activity (Table 1)”.

Comment. Please change to "This is the first antioxidant, antibacterial and phytochemical screening study for S. saman flowers. The presence of low tannin and flavonoid contents in flower (Table 4) could have contributed to medium low TPC and free radical scavenging activity (Table 1)”.

Response: Change has been made (p.19).

13. Tables

Comment: All 4 Tables are very complicated and have to be divided into Figures.

Response: Table 3 has been removed to reduce confusion. Other tables will be remained as experimental values are best presented in table form.
Conclusions
14. The conclusions has to be rewritten in order to name the concrete results of this investigation.

Response: Examples of potential plants are given (p.20).

References
15. A very long list of references (60): it is not a review.
Some of them are of old date:

Response: The manuscript has been reviewed and the number of reference has been reduced as many as we could.

The reviewed manuscript cannot be published in the present form.

Declaration of competing interests:
I declare that I have no competing interests

Reviewer 4: Ramzi Mothana

Reviewer's report:
Antimicrobial activity:
As put forward in the concept of many papers, antimicrobial extracts with MICs above 100 µg/ml are not worthwhile to be further investigated. The MIC-values described in the manuscript are far above this critical value and consequently not interesting for publication.

Response: Table 3 and the insignificant MID values have been removed. Only plant extracts that showed MID below 100 µg/ml are highlighted on p13.

Antioxidant activity may be present in all plants. DPPH assay is a really very well known and simple procedure however, screening with other in vitro-or in vivo-assays will confirm and strengthen the obtained results.

Response: We agree with reviewer’s comment. Further investigation using in vitro or in vivo-assays will be pursued on plants extracts that showed significant TPC values.

Level of interest: An article of limited interest

Quality of written English: Acceptable

Statistical review: No, the manuscript does not need to be seen by a statistician.

Declaration of competing interests: I declare that I have no competing interests.