Korespondensi paper no 6: Biotechnology Reports
BTRE

Pada Senin, Desember 23, 2019, 5:19 PM, Mohammad Faisal (Biotechnology Reports) <EviseSupport@elsevier.com> menulis:

Ref: BTRE_2019_661
Title: Bio-selective hormonal breast cancer cytotoxic and antioxidant potencies of Melia azedarach L. wild type leaves
Journal: Biotechnology Reports

Dear Dr. Sukardiman,

Thank you for submitting your manuscript to Biotechnology Reports. I have completed the review of your manuscript and a summary is appended below. The reviewers recommend reconsideration of your paper following major revision. I invite you to resubmit your manuscript after addressing all reviewer comments.

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I look forward to receiving your revised manuscript as soon as possible.

Kind regards,

Dr. Faisal
Editor
Biotechnology Reports

Comments from the editors and reviewers:
-**Reviewer 1**

-

The purpose of this paper is to test the cytotoxic and the *in vitro* antioxidant activities of wild *Melia azedarach* leaves extracts. I enjoyed reading this paper, which is straightforward, and well written, but suffers from major flaws which I will address below:

1. Regarding LC-MS analysis of ethyl acetate fraction (section 3.4), authors described the tentatively detected compounds, equivocally, omitting important details (ionization mode, parent ion fragment m/z, other characteristic fragments). Namely, the compound at Rt = 18.4 min had been identified (line 272, page 11; figure 1) as **isorhamnetin-3-*O*-rutinoside** ($C_{28}H_{32}O_{16}$); however the provided m/z value: 621 doesn't correspond to the known protonated molecular ion $[M+H]^+$ m/z 625, neither to deprotonated form $[M-H]^-$: m/z 623 of this compound (Lin and Harnly, 2007; Brito et al., 2014). In absence of further confirmation techniques (aglycone characteristic fragment $[M + H]^+$ at m/z 317, UV spectra, and/ or standard injection) the identity of this compound highly doubtful. The same remark is valid for the compound at Rt = 8.2 min, assuming that is **kaempferol dihexoside**, it should provide $[M+H]^+$ of m/z 611, not 608 (Guijarro-Díez et al., 2015). Furthermore, analysis of flavonols, contrarily to limonoids, is more appropriate in negative mode ESI (cf. Lin and Harnly, 2007).

I have also a concern regarding the presumed sterol at Rt = 17.69 (line 268, page 11). The indicated m/z value of 277 is inconsistent with the value provided by Inada et al. [11]: m/z 275.

In conclusion to this remark I will recommend:

- Mass spectra identification should be seriously revised
- Additional details with ESI experiment and identification method (section 2.8., page 7) were needed
- Further expansion of the discussion given in section 3.4., the addition of a composition table is highly recommended (see Hijaz et al. (2018), Table 2, for example). Figure 1 should also be clearly annotated accordingly.

2. This remark obviously follows from the preceding one; assuming that the ethyl acetate fraction composition is correct (section 3.4), it contains more limonoids (summing up to **38%** of total area) than alleged isorhamnetin-3-*O*-rutinoside (**18%**, according to the figure 1). While it was demonstrated that isorhamnetin inhibits the proliferation of various types of breast cancer cell (Hu et al., 2015); limonoids (Triterpenes in general) were also known of their antiproliferative activities (Zhou et al. [37]; Akihisa et al., 2013). Therefore, the discussion (lines 281-292, page 11-12) should be balanced in regard to both possibilities.

3. While labeled as "significant", the difference of seven micrograms (**102 vs 109 µg GAE/g**) between the TPC of ethanol extract (E) and ethyl acetate fraction (FE) is relatively not too meaningful to explain the difference in T47D IC_{50}, nearly four-fold lower with FE extract. Please include more detailed explanation of this observation within correlation results in section 3.5.

4. Other remarks that could enhance the paper quality were commented in the included file. In particular, I will emphasize the following points:

* Lines 80 -82 p 3: "**the methanolic extract of the plant's leaves had a better cytotoxic activity compared to the extracts of its pulps and seeds**" however according to the authors of this paper [10] : "**Furthermore, among twelve tested samples, seed kernel extract of *M. azedarach* showed the best cytotoxic activity and selectivity.**" (Refer also to the results in Table 2 of this article). This information should be carefully revised.

* Section 2.1. : Please include the sample collection period/ season etc.

* Section 2.6. : Ervina et al. [20] used this method with water extracts please give more details about how this method is adapted with other fractions, especially hexane fraction

* In abstract, discussion part and conclusion: isorhamnetin is described as an ortho-dihydroxy flavonol; this is erroneous, since isorhamnetin is 3'-methoxylated and doesn't include two OH groups in ortho-. This sentence needs to be revised.

* Table 1: Identity and Macroscopic appearance rows were superfluous

* Table 2: Please use plurals: Tannins, Flavonoids etc.

* Table 3: Please provide calibration information (equations and R^2) in "Materials and Methods" corresponding sections, not in Tables footnotes

* Figure 1 : Quality is acceptable, however identified peaks should be unambiguously annotated

References cited in this review:

Lin, L. Z., and Harnly, J. M. (2007). A screening method for the identification of glycosylated flavonoids and other phenolic compounds using a standard analytical approach for all plant materials. Journal of agricultural and food chemistry, 55(4), 1084-1096.

Brito, A., Ramirez, J., Areche, C., Sepúlveda, B., Simirgiotis, M. (2014). HPLC-UV-MS profiles of phenolic compounds and antioxidant activity of fruits from three citrus species consumed in Northern Chile. Molecules, 19(11), 17400-17421.

Guijarro-Díez, M., Nozal, L., Marina, M. L., Crego, A. L. (2015). Metabolomic fingerprinting of saffron by LC/MS: novel authenticity markers. Analytical and bioanalytical chemistry, 407(23), 7197-7213.

Hijaz, F., Nehela, Y., Jones, S. E., Dutt, M., Grosser, J. W., Manthey, J. A., Killiny, N. (2018). Metabolically engineered anthocyanin-producing lime provides additional nutritional value and antioxidant potential to juice. Plant Biotechnology Reports, 12(5), 329-346.

Hu, S., Huang, L., Meng, L., Sun, H., Zhang, W., Xu, Y. (2015). Isorhamnetin inhibits cell proliferation and induces apoptosis in breast cancer via Akt and mitogen-activated protein kinase kinase signaling pathways. Molecular medicine reports, 12(5), 6745-6751.

Akihisa, T., Pan, X., Nakamura, Y., Kikuchi, T., Takahashi, N., Matsumoto, Tokuda, H. (2013). Limonoids from the fruits of Melia azedarach and their cytotoxic activities. Phytochemistry, 89, 59-70.

-**Reviewer 2**

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1. Abstract section, sentence: the present study investigated in vitro antioxidant properties. Please add the before in vitro. As general rule in vitro should italic.

2. Please add the names of secondary metabolites obtained in abstract section. Also methodology how they obtained.

3. The antioxidant properties were obtained with IC50, what it mean? Is that a method or parameter?

4. What is this: Significant ($p < 0.05$) correlations were observed between TPC, IC50 DPPH, FRAP and IC50T47D. As far I know IC50 is obtained for the whole range of concentration tested.

5. Please add some numerical values of your results into abstract section.

6. Rewrite the abstract in a meaningful manner. Also draw a clear conclusion

7. Any word part of the title should not be included in keywords list

8. There are a number of grammatical and typo mistakes throughout the manuscript. It is very difficult for me to point out all of them. Please revise the whole paper

9. Some abbreviation have been defined but not used. e.g. antioxidant activity (AA)

10. Please add some relevant literature about plant and assays used to introduction section:

Cytotoxic, antibacterial and antioxidant activities of extracts of the bark of *Melia azedarach* (China Berry). Natural Product Research. 29 (12): 1170-1172.

Isolation of bioactive compounds from *Bergenia ciliata* (Haw.) Sternb rhizome and their antioxidant and anticholinesterase activities. *BMC Complementary and Alternative Medicine.*19: 296.

Isolation, Pharmacological Evaluation and molecular docking studies of Bioactive compounds from *Grewia optiva*. *Drug Design, Development and Therapy*. 13: 3029-3036.

Anticholinesterase, antioxidant potentials, and molecular docking studies of isolated bioactive compounds from *Grewia optiva.* International Journal of Food Properties. 22 (1): 1386-1396.

Biological and Phytochemical Evaluation of *Cotoneaster microphyllus*, *Ficus auriculata and Calotropis procera.* Latin American Journal of Pharmacy. 85 (5): 945-953.

Bio-guided profiling and HPLC-DAD finger printing of Atriplex lasiantha Boiss. BMC Complementary and Alternative Medicine. 19: 1-14.

11. Please provide voucher number of collected plant. The provided number is it or not?

12. If the plant is a wild type then please provide authorities permission letter no.

13. What was the yield of crude extract and different fractions?

14. Mathematical equation has not been provided.

15. There is need to establish the antioxidant properties with anticancer activities.

16. Introduction section the aims and objective of the study have been provided as presented in thesis. It is not a research paper style.

17. If extraction solvent ethanol was 96% then how ration was (1:10)?

18. The fractionation method needs some clarification

19. Please provide concentration ranges tested in different assays

20. No details have been provided about any assay used

21. Section 2.7: what is this? According to [22] with sligh. Please write name of author then et al and then reference number

22. Why ethyl acetate fraction has been investigated? Why not other fraction through LCMS?

23. From where the authors inferred that activity is due to inactivation of hormones? There is no detail then why claim them

24. Why the claimed compound has not been isolated through column chromatography or preparative HPLC. Is the results of LCMS are absolute?

25. There are a number compound peaks in LCMS graph but only few have been identified

26. IC50 is determined for a concentration range but in table 2 I see some thing different. Why?

27. No graph or table have provided for the determined quantities.

On Fri, Feb 21, 2020 at 8:59 AM Sukardiman Harjotaruno <maman_ht@yahoo.com> wrote:

> silahakan
>
> ----- Forwarded Message -----
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> Article reference: BTRE_2019_661
> Article title: Bio-selective hormonal breast cancer cytotoxic and antioxidant potencies of Melia azedarach L. wild type leaves
> To be published in: Biotechnology Reports
> ---
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