Reviewer's report

Title: Extra-Virgin Olive Oil (EVOO)-derived Secoiridoids and lignans: Two new families of anti-HER2 (erbB-2) phytochemicals

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Reviewer: Philippe BOUGNOUX

Reviewer's report:

Major Compulsory Revisions : none

Minor Essential Revisions : yes

1) The data presented are original and interesting. However, there is a problem in the interpretation and discussion of the data: most of the strength of the data relies on the experiments investigating the potential interaction of the polyphenolic compounds examined in this paper with HER-2. Whether these polyphenolic compounds inhibit the expression of HER-2 is clearly shown, since they modify several cell functions involving HER2. What is not convincingly addressed is whether HER-2 is their sole target, as suggested by the title of the ms., or is simply an innocent bystander of other important effects triggered by these compounds. Most of their demonstration is based on Figures 7-10-13, concerning respectively siRNA, lapatinib and trastuzumab testing. Figure 7 is supposed to present the effects of polyphenolic compounds on cell viability, proliferation or apoptosis in the presence or absence (suppressed by siRNA) of HER-2. It does not since the authors have chosen to provide a pharmacologic-like interpretation of their results, i.e.: synergism, protection or antagonism, instead of providing the raw data, which are never available. Likewise, data presented in figures 10 and 13 are pivotal to stress the type of interaction of the polyphenolic compounds with HER-2, i.e.: extracellular domain investigated through the effect of Trastuzumab, or tyrosine kinase activity of the receptor investigated through the effect of Lapatinib. Again, it is impossible to appreciate the scientific strength of these results since the data are transformed into a similar interpretation. This makes the strength of the original data implicit while they could easily be explicated (in a presentation similar to that used in Figure 12). Therefore, if the authors are willing to put a high emphasis on these particular data, then they should provide the raw data on which they built their interpretation. If they believe that this is not needed, then they should weaken the emphasis they put on to this part of the manuscript.

2) Other data are generally sound, with the exception of the results presented in the electronic supplement in figures I and II. The data documenting the effects of NAC or Trolox on EVOO polyphenolic compounds actions on cancer or control cell are not provided. Therefore, the hypothesis that the effects of polyphenolic compounds on cells are mediated through an oxidative stress mechanism is not substantiated.
3) Limitations of the work are not clearly stated. The experimental system used (i.e.: transformed cell lines) is not appropriate to investigate polyphenolic compounds effects on HER-2 positive breast cancer incidence. Thus, considering the results, this proposition is an overstatement.

4) Title and abstract have to be modified according to previous comments.

Concerning the other parts of the ms. there are few minor essential revisions to underline:

5) Methods are appropriate and well described, with the exception of the methods used to investigate the role of the oxidative stress and that of the proteasome, which are lacking.

6) Several criticisms relate to the style and organization of the manuscript. The amount of data provided is abundant and the data are diluted by repetitions (as an example, the same information is provided under different forms both in the Material and Methods section and the Results section). A more concise style will improve the quality of the paper.

8 Other comments:
Figures labelling:
- “Figure x “ is repeated twice in all figures presented. This should be corrected.
- Information on polyphenolic compounds concentrations used is lacking in figures 5-6-12-13.
- Cell type is lacking in figures 3-4-8-12.

Since results presented in figure 7 left panel and in figure 10 upper panel are experiments quality control, they could be presented in the electronic supplement to help make the ms. more concise.

Statistics in figure 12: “Fraction x (+) / MG-132 (-)” has to be compared to “Fraction x (+) / MG-132 (+)”.

Legends: “3.) 1µM MG-132 (2h) # EVOO...” is wrong and should read “3.) 1µM MG-132 (2h) # EtOH...”.

Discretionary Revisions:
Some abbreviations are lacking (such as ELISA, IMEM, FSB, ATCC,OD...).

Other comments:
The authors clearly acknowledge any work upon which they are building, both published and unpublished.

In conclusion, this article is of importance in the field of breast cancer biology. However, the authors have to respond to the “Minor Essential Revisions” comments and modify their manuscript accordingly to make it convincing.
Level of interest: An article of importance in its field

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