Author's response to reviews

Title: Antioxidant genotoxic and antigenotoxic activity of Daphne gnidium leaf extracts

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Author's response to reviews: see over
Dear Editor,

I have the pleasure to submit for publication to your journal, our revised paper entitled:

**Antioxidant, genotoxic and antigenotoxic activity of *Daphne gnidium* leaf extracts**

By: Fadwa Chaabane, Jihed Boubaker, Amira Loussaif, Aicha Neffati, Somaya Kilani-Jaziri, Kamel Ghedira, Leila Chekir-Ghedira

I confirm that this manuscript was submitted solely to this journal and is not published, in press, or submitted elsewhere. I have also prepared my paper and files in accordance with the journal's style and format requirements and all co-authors agree to its publication.

We are willing this paper to be considered for publication in your journal as soon as possible.

Waiting for hearing from you,

Best regards

Pr. Chekir-Ghedira L.
Responses to Reviewers

Responses to Reviewer 1

Methods

As asked by reviewer 1:

- We have listed the different concentrations used for each sample in each method in the corresponding section method.
- We indicated the room temperature.
- We indicated to how many hours this culture corresponded.

Results

- Plant materials were screened for the presence of tannins, flavonoids and coumarins by thin layer chromatography (TLC) on silica gel. Coumarins were detected under UV (366 nm) thanks to their blue fluorescence. However, we have used quantitative analysis only for polyphenols, flavonoids and tannins.
- The sentence “According to …..as genotoxic if the IF exceeds 2” was transferred to materials and methods section.
- The sentence “Dose of 5μg/essay…..SOS response” was transferred to materials and methods section.
- The sentence “the test implements two…….spectrophotometrically at 560nm” was transferred to materials and methods section.
- We changed 1 mg/ml by 0.5 mg/ml.
- We added a figure (Fig.3) which illustrates the pro-oxidant effect of chloroform and petroleum ether extracts.
**Responses to Reviewer 2**

**Title**
- As asked by reviewer 2 we changed the title of the manuscript to “Antioxidant, genotoxic and antigenotoxic activity of *Daphne gnidium* leaf extracts”

**Background**
- We supported the statement “In some countries, governments encourage the use of indigenous forms of medicine rather than expensive imported drugs”. With relevant reference.

**Methods**
- We indicated the conditions of temperature and relative humidity where the leaves were dried and stored.
- Yes we used distilled water.

**Results**
- We cited the IFs in the text.
- The axis Y in figure 1 was reduced to 100% of inhibition instead 120%.
- We changed the color.

**Responses to Reviewer 3**
- The reviewer said “that our study is largely confirmatory”:
It is true for the antioxidant activity of this plant, nonetheless, this activity was only investigated against linoleic acid oxidation (Deiana et al., 2003). Whereas we prospected its antioxidant capacity against superoxide anion and uric acid formation using enzymatic and non enzymatic systems.
Whereas concerning the genotoxicity / antigenotoxicity studies, our work seems to be the first which allude to such activity for this plant.
- The reviewer said that “genotoxicity and anti-genotoxicity tests are completely different “
In fact, the only difference between the two tests is the addition of the toxicant (the nitrofurantoin) against which the antigenotoxic capacity of the plant extract is evaluated. In both experiments we used the same concentrations of extract and the same number of bacterial cells. These methods are largely described in literature (Quillardet and Hofnung, 1985; Kaur et al., 2009; Hayder et al., 2004)

- The reviewer said that “authors used old references to evaluate the presence of tannins flavonoids and coumarins in plant extracts” we changed them by more recent ones:
  

  We changed Harbone (1974) by Raman (2006)


- The reviewer said that “authors did not consider the period of year the latitude....”

Concerning the period of year we already specify that collect was done in November 2009 which corresponds to the period of bloom as far as we used this organ (blooms) in further studies. In fact to be able to compare the results according to the studied organ, we choose to collect them at the same period. Concerning the latitude, plant leaves were collected from Bizerte, a region which is situated in the north of Tunisia (latitude: 37.27, height: 22)

- We added in the discussion details that indicate compounds which may be responsible of the observed activities:

Page 14, line 342 to line 352:

“All these results confirmed our hypothesis that polyphenols contained in the tested extracts are responsible of their antigenotoxicity. In fact, our chemical study let to the identification of apigenin-7-glucoside from methanol and TOF extracts by HPLC analysis (data not shown). Zaabat et al. (40) showed, by SOS chromatostest, that this compound prevent the genotoxicity produced by nitrofurantoin. The antigenotoxicity of ethyl acetate and chloroform extracts should be ascribed to the presence of terpenes (beta amyrin acetate) and lignans (dihydrosesamin) we detected in the two mentioned extracts. In fact, Nikolic et al. (41)
reported that plant terpenes exhibited antigenotoxic activity. On the other hand Siddique et al. (42) demonstrated that a phenolic lignan (nordihydroguaiaretic acid), possesses an antigenotoxic potential against chlormadinone acetate induced genotoxic damage in mice bone-marrow cells.”

**Page 16 to page 17, line 403 to line 407:**

“In fact, we have identified, by HPLC, the presence of daphnetin in the methanol and TOF extracts (data not shown). It was reported that this coumarin have a radical scavenging and anti-lipid peroxidation effect (57). As we have identified the presence of apigenin-7-glucoside in TOF extract and luteolin-7-glucoside in the methanol extract (data not shown),”