Reviewer’s report

Title: Antioxidant capacity of chewing stick miswak Salvadora persica

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Reviewer: Azza M. Abdel-Aty

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The search for novel natural antioxidants of plant origin has ever since increased. It is not known which constituents of plant protect us from various diseases; therefore, antioxidants appear to play a major role in the protective effect of plant medicine. The present study evaluate the antioxidant activities (in vitro) of the methanol extract of chewing stick miswak Salvadora persica. In general the methods and results show relevant information although some points need to clarify more. The manuscript need to be revised before publication according to comments are pointed out below.

Specific Points:

1- In Background:
   a- You focused only on the uses of the miswak and its benefits for oral hygiene as anti-fungal and anti-bacterial. You must be add the family mane, genus, species and the distribution of miswak, in addition, its uses in folk medicine for treatment several illnesses.
   b- The aim of the study is very short and not clear (page 5, lines 92-94).

2- In materials and methods
   a- Plant material
      (Miswak Salvadora persica L. (Salvadoraceae) root was purchased from local market of Jeddah, Kingdom of Saudi Arabia) page 10, lines 198-199. Must be bring it from well known source and be sure classification, the plant material was botanically identified by Dr…………., from Department of Plant Sciences of ……..
   b- Preparation of solvent extracts
      • Add the weight of the dried miswak root.
      • Why use the root of miswak as it is without coarsely powdered it?
      • Why use 24-h incubation, not less or more? Where, in many previous studies extraction incubation time more than 48 hours.
      • Add how to calculate the yield.
• After this title (Preparation of solvent extracts) you should be add new title (chemicals) which uses in this study.

c- ABTS radical cation decolorization assay, page 11, 221 to 245.
• This assay should be rewritten, avoid unnecessary details.
• In this sentence (The ABTS•+ solution was diluted to give an absorbance of 0.750 ± 0.025 at 734 nm in 0.1 M sodium phosphate buffer pH 7.4) why the dilution was performed by 0.1 M sodium phosphate buffer pH 7.4? While, in the original method according to (Re et al., 1999) the dilution performed with 50% methanol.
• In the original method according to (Re et al., 1999), the decrease in absorbance was measured exactly one minute after mixing the solution, then up to 6 min, in this manuscript why the absorbance was recorded 5 min after mixing.
• In line 238-239, (calculated using the following equation: ABTS•+ scavenging (%) = (1- AS/AC) x 100)
Should be changed to:
• (ABTS•+ scavenging (%) = [(control absorbance- sample absorbance)/ (control absorbance)] x 100, Like all previously written equations.

d- Phosphomolybdenum complex assay page 12.
• Line 251, (Sample solutions were combined in an Eppendorf tube) the volume of the sample should be add.
• Line 255, 256, (the absorbance of aqueous solutions of each was measured at 820 nm) are you sure of the absorbance measurement at 820 nm, although, in the original method according to (Prieto et al., 1999) the absorbance measurement at 765 nm.
• Line 257, why the antioxidant activity was expressed as the absorbance of the sample? Although, in the original method according to (Prieto et al., 1999), the antioxidant capacity was estimated using following formula: Antioxidant effect (%) = [(control absorbance-sample absorbance) / (control absorbance)] ×100. You should be re-calculate the Antioxidant effect (%) by the above equation and change the obtained results.

e- GC-MS analysis, page 12, lines 262-265.
• )The temperature program were set as follows: 50ºC hold for 5 min, raised at 10ºC /min to 250 ºC, and hold for 10 min. The injector and detector temperatures were set at 280ºC. The ion source and interface temperatures were set at 200 and 250ºC, respectively. (this part should to be more clarified.

Determination of antioxidant enzymes
f- Preparation of crude extract, page 13.
• Line 275-276 (One g of miswak was homogenized with 20 mM Tris–HCl buffer, pH 7.2 contained 0.1 M NaCl) which part of miswak, and why did you use this buffer??
• Why did you use the root of the miswak, while in your previous study (Mohamed et al., 2012) proved that the miswak stem had most peroxidase activity among all the miswak parts.

• Material and methods section should be preceded the results section, according to the recent issues of the journal. And in abstract too.

3- Results and discussion
• Page 6, line 99-104, why you use the weight of miswak for determination the best yield of solvent extracts? The antioxidant activity should be measure for different solvent extracts from dried material.
• Line 118, (4.8 µg) should be correct to (4.8 µg / ml).
• Line 119, (The correlation coefficient between crude methanol extract and DPPH scavenging activity was found to be 0.97.) add your comment on this results as strong positive correlation and on the similar results.
• Line 124, what is (the trolox equivalent)?
• More discussion about (ABTS) assay and its mechanism of action.
• Line 137, the sentence (phosphate/Mo (V) complex) why this sentence in abbreviation form.
• Page, 9 line 183-184, (A Caribbean copper plant peroxidase from the latex of Euphorbia cotinifolia was studied) why you use this example on the presence of peroxidase in plant, whereas, the peroxidase common enzyme in almost the plants.
• Your discussion about the antioxidant enzymes in this manuscript is very short.

4- Conclusion, page 9.
• Line 193, (The synergistic actions of antioxidant compounds and antioxidant enzymes make miswak is a good chewing....), how proved the synergistic actions of antioxidant compounds and antioxidant enzymes in miswak, you did not isolate or purify them from each other.

5- List of legends, page 20.
• Fig 1. (Correlation between the total content of miswak crude methanol extracts and their antioxidant capacity as determined by DPPH assay.) Should be change to (Correlation between different concentrations of miswak crude methanol extract and their antioxidant capacity as determined by DPPH assay.) and the same changes will be carried out in Fig. 2, and Fig.3.

6- Tables, page 21.
• Table 1, measure the antioxidant activity of each miswak crude solvent extract.

7- Figures:
• Fig 1. The legend at X-axis should be change from (µg Crude methanol extract) to (Concentration (µg/ml). and the same changes should be carried out in Fig. 2,
and Fig.3.

• Fig.3. The legend at Y-axis should be change from (O.D. at 820 nm) to (phosphomolybdate % scavenging) after calculate the phosphomolybdate scavenging activity %.

**Level of interest:** An article of importance in its field

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

**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’