Author's response to reviews

**Title:** Evaluation of antimicrobial, antimitogenic and radical scavenger activities of polar extracts from (Tunisian) Acacia salicina (Lindl) leaves

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**Author's response to reviews:** see over
Editor in chief of BMC Complementary and Alternative Medicine

Dear Editor,

I have the pleasure to submit the revised manuscript for publication in your journal: BMC Complementary and Alternative Medicine, the revised version of our paper entitled:
« Evaluation of antimicrobial, antimutagenic and radical scavenger activities of polar extracts from (Tunisian) Acacia salicina (Lindl) leaves» by: Jihed Boubaker, Hedi Ben Mansour, Kamel Ghedira, Leila Chekir Ghedira. Referenced: MS ID 3930931815928668
Responses to Reviewer Alexandra Acco

- I correct the references list
- I correct the legend of Table
- With proposal from reviewer, we complete the tables and figures with adequate statistical study”

- The reviewer said: “In discussion (item 3.1) the authors mentioned the effects of A. salicina upon Salmonella. However, there are no comments about the comparison of A. salicina extracts with positive control ampicillin. Clearly ampicillin has lower MIC value, 32-fold lower concentration compared to TOF extract and 641-fold compared to metanolic extract. The authors may mention these huge differences in the MIC values.”

I answer: “The purpose of this study is to determine whether plant extracts possess an antibacterial effect or not, even if these extracts are not as active as the antibiotic because this study open the way to find a modulating effect between extract and antibiotic.”

- The reviewer said: “Despite the group has been published similar articles in the last years, there is no comparison of the presented results with effects of A. salicina previously published, concerned to different fractions of this plant or other methods used for the same proposal (antimutagenic and antioxidant actions). The authors should consider these aspects in the discussion.” I added in the end of discussion this section: “The major and most difference between the all papers published by Bouhlel et al. (36) (44) and the presence paper is that A. salicina described by Bouhlel et al. was collected in the Monastir region (center of Tunisia) however, our plant, was collected from the Arid Region Institute (IRA) situated in the south east of Tunisia. The chemical analysis of the extracts obtained from the two plants revealed that the extracts composition is totally deferent. This explains the behaviour difference of the two plants against same biological activities. On the other hand the paper of Mansour HB et al. (2007) (29) describes the antigenotoxic effect of extracts obtained from Acacia salicina using the SOS chrometest with Escherichia coli PQ37. In order to confirm the protection power of Acacia salicina extracts against genotoxic effect we should employed many assays. For this, in the present paper, antigenotoxic activities are carried out by Ames
assay using *Salmonella typhimurium* TA98 and TA102, the difference between the superoxide anion scavenger activity observed in the present paper and that described by Mansour et al. (2007) (29). The superoxide scavenger activity was carried out by an nonenzymatic generating system (NBT/riboflavin) and an enzymatic xanthine/xanthine oxidase system. The enzyme xanthine oxidase catalyzes the oxidation of xanthine to uric acid. During this reaction, molecular oxygen acts as an electron acceptor, producing superoxide radicals according to the following equation:

\[
\text{Xanthine} + \text{O}_2 \rightarrow \text{uric acid} + \text{O}_2^- + \text{H}_2\text{O}
\]

**Xanthine oxidase**

The inhibition of xanthine oxidase activity was measured according to the increase in absorbance at 290 nm (uric acid) and 530 nm (superoxide anion). The influence of the *Acacia salicina* leaf extracts on XOD activity evaluated by uric acid and superoxide anion formation as the final products. In fact, in the paper of Mansour et al. (2007) (29) we followed only the uric acid evolution in the presence of *Acacia salicina* extracts. However, in this paper the superoxide formation was quantified by spectrophotometer.

- With proposal from reviewer I completed the article with a general conclusion of the article.

**Conclusion**

The experiments described above demonstrated the interaction between secondary metabolic composition of extracts and each radical and strain and the polar extracts from (Tunisian) *Acacia salicina* leaves exhibited significant potent radical scavenger, antimicrobial and antimutagenic activities. This work paves the way for studying this medicinal plant in the induction of apoptosis in cancer cells line.

- We tried to address the grammatical and typographical errors as asked by the reviewer.
**Responses to Reviewer** Dario Kremer

The reviewer said « A set of tubes containing only agar and microbial suspension served as the negative control.’ Where are these results » I answer” the results obtained is only a bacterial layer”.

The reviewer asked the results of the statistical study. I agree with the reviewer are the results have been signed in the result in Tables.

The reviewer said “Conclusion is missing at the end of section Results and discussion. Please, make few sentences such as: 'In conclusions,… ”’ I agree with the reviewer and I added at the end of section Results and discussion the section entitled Conclusion: The experiments described above demonstrated that polar extracts from (Tunisian) *Acacia salicina* leaves exhibited significant potent radical scavenger, antimicrobial and antimutagenic activities. This work paves the way for studying this medicinal plant in the induction of apoptosis in cancer cells line.

Minor Essential Revisions

Title:
Page 1: I add 'Lindl.' after 'salicina'

Affiliations:
Page 1: I change 'boubaker' into 'Boubaker' and ‘chekir’ into ‘Chekir’

Abstract:
Page 2: line 1: I explain abbreviation 'TOF' “Total Oligomeres Flavonoids”
Page 2, line 2: I add 'Lindl.' after 'salicina'
Page 2: I explain abbreviation 'NOPD' “4-nitro-o-phenylenediamine”
Page 2: I change type of brackets in '(a)' to be the same like in Material and methods
Page 2: I explain abbreviation 'TEAC' Trolox Equivalent Antioxidant Capacity
Page 2: I use always 'ABTS+•' and always I put '•' in bold (to be consistent in all text)

1. Introduction
Page 3: paragraph 2, line 5 I put ‘, ’ after '[5],' 
Page 3: paragraph 2, line 8: I change bold '.' in normal after [9].
Page 3: paragraph 2, line 14: Please, explain sentence 'Traditional medical uses of Acacia in north Tunisia are somewhat different.' It could help others investigations. I replace this sentence by “Whereas in north Tunisia it is used for the treatment of diarrhoea and rheumatisms”

2. Material and methods
2.2. Plant materials

Page 4: paragraph 1, line 2: I add space in 'Chaib (14)'

Page 5: paragraph 1, line 5: I use 'hour' instead of 'hr'

2.4. Bacterial strains

Page 5: paragraph 1, line 3: I change '('14)' into '('15)' and correct references number in all manuscript

Page 5: paragraph 1, line 7: I change '('15)' into '('16)' and correct references number in all manuscript

2.5. In vitro antimicrobial activity

Page 6: paragraph 1, line 1: I change 'Salicina' into 'salicina'

Page 6: paragraph 1: I change 'gram' into 'Gram'

Page 6: paragraph 1: I correct 'entretidis'

Page 6: paragraph 1: I explain how the number of microbes were identify “I answer “The bacterial concentration was determined from calibration cure (optic density versus number of bacteria). Then the number of bacteria was determined by preparing bacterial culture with different optic densities, 10µl of each culture bacteria were plated on substance-free Muller-Hinton agar plates and further incubated at 37°C for 24 hour.

Page 6: paragraph 1: I change letter 'x' into symbol '#'

2.6. Radical-scavenging activity on ABTS**

Page 6: paragraph 1, line 1: please, I explain abbreviation of 'ABTS' 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt

Page 7: paragraph 1, line 13: please, omit space before '2-carboxylic'

2.7. Superoxide anion Scavenging Activity

Page 8: paragraph 1, I explain abbreviation 'X/XOD' "xanthine/ xanthine oxydase"

Page 8: paragraph 2: I add 'of' before 'EDTA' and I explain abbreviation 'EDTA' "Ethylenediaminetetraacetic acid"
Page 8: paragraph 2: I add 'of' before 'XOD'
Page 8: paragraph 2: I correct '37 °C'

2.9. Salmonella-microsome assay
Page 9: paragraph 1: I correct '37°C'

3. Results and discussion
3.1. Antimicrobial activity
Page 11, paragraph 1: I change ',' into '.' in '0,125'
Page 11: I put 'S. aureus' in Italic
Page 11: I change ',' into '.' in '0,225'
Page 12: I change 'sp.' into 'spp.'

3.2. Antioxidant activities
Radical-Scavenging activity on ABTS.
Page 12, title: I omit '.' after 'ABTS'
Effects on superoxide anion generating system
Page 13, paragraph 1, line 5: I put 'Figure 1' after 'each extract'

3.3. Mutagenic and antimutagenic activities
Page 14, paragraph 1: I change ',' into '.' in '93,5%'
Page 14, paragraph 2: I add space in 'S. typhimurium TA98'
Page 17, paragraph 1, line 1: I put 'S.' in Italic
I use long (Ref. no. 11, 12)
Ref. no. 2: I add spaces in 'J. Pak. Med. Assoc.'
Ref. no. 10: I change 'Australia' into 'Australian'
Ref. no. 12: I add space after '(12)'; I put 'Phy' and 'Curr' in Italic; I change 'Curr.' into Normal (not Bold)
Ref. no. 13: I add space after '(13)'
Ref. no. 15: I put 'Mutat' in Italic
Ref. no. 16: I put 'Salmonella' in Italic
Ref. no. 17: I add space after '(17)'; I put 'Salmonella' in Italic
Ref. no. 18: I put 'Salmonella' in Italic
Ref. no. 19: I put 'Myrtus communis' in Italic
Ref. no. 21: I put 'Rhamnus alaternus' in Italic
Ref. no. 22: I add space after 'Biol.'
Ref. no. 26: I delete space in 'M M'; correct 'Annasl'
Ref. no. 29: I put 'Acacia salicina' in Italic
Ref. no. 30: I put 'Caesalpinia pulcherrima' in Italic
Ref. no. 34: I delete '.' in 'E.C.D.'
Ref. no. 36: I make correction in 'ABTS+ '
Ref. no. 37: I put 'Acacia salicina' in Italic; I add space after 'extracts'
Ref. no. 39: I make correction in 'flavonoïds'
Ref. no. 41: I add space after '540'
Ref. no. 43: I omit 'and'

Discretionary Revisions
I change 'ml' into 'mL'

3. Results and discussion
3.1. Antimicrobial activity
Page 10, line 1: Please, I add 'leaves' after 'A. salicina'

I connect Table 1 and Table 2 into one table.

- We tried to address the grammatical and typographical errors as asked by the reviewer.
Responses to Reviewer  Hui Meng Er

- The reviewer said “It is claimed that the extract components cannot block all sites of DNA from electrophilic attack. Please explain.” I answer “what we want to explain the term partially what the effect of the extracts cannot be due to a single way, but by one or more separate ways and we replace the sentence “that extracts components may interact partially with target tissue DNA; in turn, they can’t block all sites of DNA to electropholic attack by reactive mutagenic moieties.” By “the protective effect of the extracts against the tested mutagens may probably be exerted by three ways; the first, is that the plant extracts may adsorb the mutagen in a way similar to the carcinogen adsorption which has been associated with chemical component; the second, is that the extracts could induce DNA glicosylase enzymes which are capable of repairing alkylating DNA bases, finally the reductive ability of the samples assessed in this study suggests that extracts were able to donate electron to free radicals, making the radicals stable and unreactive (38).”

- The reviewer said ”Please provide a plausible explanation for the toxic effects of the extracts against TA102 strain.” I answer “This effect may be ascribed to the formation of a complex between the mutagenic agent (2-AA) and extract components when large excess of each extract was added to the assay system, which may exhibit a mutagenic effect toward a specific Salmonella strain, could be explained by the inhibition of the penetration through the cell membrane at high doses of extracts or molecules which are implied in the mutagenic inhibitory effect towards mutagenic agent (38).”

- The reviewer said ”In section 3.3 last paragraph last sentence, please elaborate on the statement that the complexes formed between the extracts and B(a)P prevent their penetration through bacterial cell wall. Please propose studies to substantiate this statement.” I added the study of Bouhlel et al (44).

- The reviewer said “There should be a conclusion section to comment on the significance of the findings in this study, especially on the mutagenic activities of the extracts.” I agree with the reviewer and I added at the end of section Results and discussion the section entitled
Conclusion: The experiments described above demonstrated the interaction between secondary metabolic composition of extracts and each radical and strain and the polar extracts from (Tunisian) *Acacia salicina* leaves exhibited significant potent radical scavenger, antimicrobial and antimutagenic activities. This work paves the way for studying this medicinal plant in the induction of apoptosis in cancer cells line.

-The reviewer said” In section 2.9 sentences should not start with numbers (eg 100 uL)”. I write the sentence and I start with “We take 100 ul ….”

The reviewer said “First paragraph Please provide diagram to show the biphasic reaction kinetics “ In fact what we mean by this phrase is the correlation, and I change the sentence “the biphasic reaction kinetics” by “the correlation”

I connect Table 1 and Table 2 into one table.

Section 3.2
Radical-Scavenging activity on ABTS
I connect Table 1 and Table 2 into one table and I corrected the citations of Table 2 in the section Radical-Scavenging activity on ABTS Effects on superoxide anion generating system.

I referred Figure 1 at the end of fifth sentence in section Effects on superoxide anion generating system.

- We tried to address the grammatical and typographical errors as asked by the reviewer.
Reviewer: Eduardo Madrigal-Bujaidar

I think the changes made to the Results and Discussion section provide more claret and do not need to be separate in two different sections. But if you and editor see otherwise, I can be separate in two sections.

In abstract I give the full names of abbreviations cited.

**Total Oligomeres Flavonoids (TOF)**
**methylmethane sulfonate (MMS)**
**Benzo[a]pyrene (B(a)P)**
**Trolox Equivalent Antioxidant Capacity (TEAC)**
**2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS)**
**4-nitro-o-phenylenediamine (NOPD)**

We changed the sentence “Besides, it has been found these compounds in their natural formulation are more active than their isolated form” by the “Besides, it has been found these chemical families are more active by acting synergistically”

In the discussion, we give the differences between this study and with respect to those previously published on the matter. So we added in end of discussion section “The major and most difference between the all papers published by Bouhel et al. (36) (44) and the presence paper is that A. salicina described by Bouhlel et al. was collected in the Monastir region (center of Tunisia) however, our plant, was collected from the Arid Region Institute (IRA) situated in the south east of Tunisia. The chemical analysis of the extracts obtained from the two plants revealed that the extracts composition is totally deferent. This explains the behaviour difference of the two plants against same biological activities. On the other hand the paper of Mansour HB et al. (2007) (29) describes the antigenotoxic effect of extracts obtained from *Acacia salicina* using the SOS chrometest with *Escherichia coli PQ37*. In order to confirm the protection power of *Acacia salicina* extracts against genotoxic effect we should employed many assays. For this, in the present paper, antigenotoxic activities are carried out
by Ames assay using *Salmonella typhimurium* TA98 and TA102, the difference between the superoxide anion scavenger activity observed in the present paper and that described by Mansour et al. (2007) (29). The superoxide scavenger activity was carried out by an nonenzymatic generating system system (NBT/riboflavin) and an enzymatic xanthine/xanthine oxidase system. The enzyme xanthine oxidase catalyzes the oxidation of xanthine to uric acid. During this reaction, molecular oxygen acts as an electron acceptor, producing superoxide radicals according to the following equation:

\[
\text{Xanthine} + \text{O}_2 \rightarrow \text{uric acid} O^\cdot_2 + \text{H}_2\text{O}_2
\]

The inhibition of xanthine oxidase activity was measured according to the increase in absorbance at 290 nm (uric acid) and 530 nm (superoxide anion). The influence of the *Acacia salicina* leaf extracts on XOD activity evaluated by uric acid and superoxide anion formation as the final products. In fact, in the paper of Mansour et al. (2007) (29) we followed only the uric acid evolution in the presence of *Acacia salicina* extracts. However, in this paper the superoxide formation was quantified by spectrophotometer.

Material and Methods
Page 6: paragraph 1: I change 'gram' into 'Gram'
In subsection 2.6, I deleted ‘(%)' after ‘percent inhibition’

Results and discussion

We delete the sentence “In Canada, the cost to treat the foodborne disease due to meat and meat products contamination is estimated to $500 millions/an (34).”

On the proposal of reviewer we deleted the paragraph of subsection 3.2.

Radical-Scavenging activity on ABTS

The reviewer said “In line 5 of the first paragraph one sees written, “(reference)”. What does this mean?? “ I answer “It's just an editorial mistake and we delete (reference)”
The reviewer said “The explanation shown at the end of subsection 3.3 (second paragraph) is unclear. Which molecules are the authors referring to. Are they the same ones involved in the useful effects? How can the mutagenic effect be explained?” I answer “Concerning the relation between toxic effects of extracts of TA102 strain and the different chemical families that make up these extracts we giving us the following explanation and will be added to the text in section 3.3 fourth paragraph 4th sentence “This effect may be ascribed to the formation of a complex between the mutagenic agent (2-AA) and extract components when large excess of each extract was added to the assay system, which may exhibit a mutagenic effect toward a specific Salmonella strain, could be explained by the inhibition of the penetration through the cell membrane at high doses of extracts or molecules which are implied in the mutagenic inhibitory effect towards mutagenic agent (38).”

The reviewer said ” The statement in subsection 3.3, starting at Teel (37) (fourth paragraph) is also very general and vague. The authors should revise it and make it more precise.” I replace the sentence “that extracts components may interact partially with target tissue DNA; in turn, they can’t block all sites of DNA to electropholic attack by reactive mutagenic moieties.” By “the protective effect of the extracts against the tested mutagens may probably be exerted by three ways; the first, is that the plant extracts may adsorb the mutagen in a way similar to the carcinogen adsorption which has been associated with chemical component; the second, is that the extracts could induce DNA glicosylase enzymes which are capable of repairing alkylating DNA bases, finally the reductive ability of the samples assessed in this study suggests that extracts were able to donate electron to free radicals, making the radicals stable and unreactive (38).”

- The reviewer said ” The authors should also present stronger support for their hypothesis shown at the end of subsection 3.3.” I answer “ following the changes made to the discussion We explained above why the extract are mutagenic against the strain TA102 and will be added to the text in section 3.3 fourth paragraph 4th sentence “This effect may be ascribed to the formation of a complex between the mutagenic agent (2-AA) and extract components when large excess of each extract was added to the assay system, which may exhibit a mutagenic effect toward a specific Salmonella strain, could be explained by the inhibition of the penetration through the cell membrane at high doses of extracts or molecules which are implied in the mutagenic inhibitory effect towards mutagenic agent (38).”
- We tried to address the grammatical and typographical errors as asked by the reviewer.