Author's response to reviews

Title: Evaluation of antimicrobial, antimitagenic 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 resubmit 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: **********

**Responses to Reviewer**

Page 2, line 2, I delete “leaves” after Lindl and added “the leaves of” after from

Page 2 line 11, I replace « activities was » by « studies”

Page 2 line 13, I added Likewise,

Page 3 line 1, I delete for

Page 3 line 6, I replace “the latter” by “these plants”

Page 3 line 12, I delete activity in anti-inflammatory activity

Page 3 line 15 I remove the “uses of Acacia differ” by the “use of Acacia differs”
Page 3 line 16 I remove « region of the country” by “the country region”

Page 3, last paragraph, I delete ”As well as other Acacia species, Acacia salicina showed a high level of salt tolerance (10).”

Page 3, last paragraph, I replaced “are” by “were” and “described” by “reported”

Page 4, first paragraph, I delete Besides, it has been found that compounds in their natural formulations are more active than their isolated form (12) (13).

Page 4, first paragraph, I replace “We demonstrate” by “Our study revealed”

Section: Results and discussion,

Section Antimicrobial activity

Line 1, I remove leaves by leafs.

Line 4, I remove “Minimum Inhibitory Concentration (MICs) values varied from 0.0625 to over 10 mg/ml, and Minimum Bactericidal Concentration (MBCs) values varied from 0.125 to over 10 mg/ml” by “Minimum Inhibitory Concentration (MICs) values ranged from 0.0625 to over than 10 mg/ml, and Minimum Bactericidal Concentration (MBCs) values ranged from 0.125 to over than 10 mg/ml”

Line 7, I remove “displayed strong activity” by “displayed a strong activity”

Line 11, I remove “used as positive control” by “used as a positive control”

Line 12, I remove “two orders of magnitude” by “twice”

Line 13, I delete “In contrast, compared to the other bacterial strains”

Line 13, I added strain in the end of sentence.
Line 14, I remove “TOF extract was more active against all bacterial strains” by “TOF extract was the most active one, against all the tested bacterial strains”

Page 5, line 1, I remove “Our results indicate that Gram-positive bacteria are more sensitive to the tested extracts of A. salicina than Gram-negative ones” by “Our results indicate that Gram-positive bacteria are more sensitive to the antimicrobial effect of A. salicina extracts than Gram-negative ones”

Page 5 line 8, I remove “and the European Union also reported more than 100,000 cases” by “The European Union reported more than 100,000 cases of salmonellos (15)”

Page 5, line 15, I remove “outbreaks, cases, and deaths” by “outbreak cases and deaths”

**Section Antioxidant activities**

I remove the title “Radical-Scavenging activity on ABTS” by “Radical-Scavenging activity against ABTS$^+$”

Line 1, I removed ”by” by ”with”

Line 4, I removed “A potential activity was noted at different tested concentrations of all extracts studied (table 2)” by “A potential activity was noted at the different tested concentrations of all studied extracts (table 2)”

Line 5, I added “the positive control,“

Line 6, I replaced “greater” by “higher”

Page 6, line 1, I removed ” Some authors have described the correlation between the ABTS$^+$ and some polyphenols in food (34) (35).” by “Some authors have described the capacity of polyphenols to exert an antioxidant effect against ABTS$^+$ (17) (18).“
Line 3, I removed “extracts tested” by “tested extracts”

Line 9, I replaced “of a sample” by “from the sample,”

Line 13, I removed “which largely exceed 1mM the TEAC value of the positive control (Trolox)” by “which largely exceed the TEAC value of the positive control Trolox (1mM)”

Section Effects on superoxide anion generating system

Page 7, line 2, I replace “while these compounds are known to have an antioxidant activity, as described by Bouhlel et al. (36)” by “which are known to have an antioxidant capacity, as described by Bouhlel et al. (36)”

Mutagenic and antimutagenic activities

Line 10, I remove “the mutagenic effect is observed with all tested extracts in the presence of S. typhimurium TA98 strain and aqueous extract in S. typhimurium TA 102 strain” by “a mutagenic effect is observed with all the tested extracts in the presence of S. typhimurium TA98 strain at the two highest tested dose, and only with aqueous extract at the highest tested dose in S. typhimurium TA 102 assay system”

Line 15, I remove “antimutagenic activity of these extracts” by “antimutagenic capacity of A.salicina extracts”

Line 17, I remove “of positive mutagens” by “induced by the afore mentioned toxicants,”

Line 19, I remove “The inhibitory effect of different extracts from A. salicina on the mutagenicity of positive mutagens, using the plate incorporation assay is illustrated in tables 4 and 5. As shown in table 4, all extracts prepared from A. salicina were effective in reducing mutagenicity, in the S. typhimurium TA 98 and TA 102 strains, induced respectively by NOPD (10 µg/plate without S9) and MMS (2.5 mg/plate), a direct-acting genotoxins” by
“The inhibitory effect of different extracts from *A. salicina* on the mutagenicity induced by the aforementioned toxicants, using the plate incorporation assay, is illustrated in tables 4 and 5. As shown in table 4, all extracts prepared from *A. salicina* were effective in reducing the mutagenicity induced by NOPD (10 µg/plate without S9) and MMS (2.5 mg/plate), a direct-acting genotoxins, in respectively *S. typhimurium* TA 98 and TA 102 assay systems.

Page 8 line 1, I replaced “All tested extracts” by “All the tested extracts”

Page 8 line 6, I deleted “the high”

Page 8 line 14, I replace “that the extracts could in duce” by “that extracts could induce”

Page 8 line 21, I replace “within” by “in”

Page 8 line 21, I replace “respectively, 66.3%, 65.1% and 89.5% in the presence of the lowest dose (50 µg/plate)” by “in a dose dependent manner”

Page 9 line 1, I replaced “However, when tested at the same doses (50, 250 and 500 µg/plate) all extracts, mixed with 2-AA, showed an influence of cell viability and exhibited a toxic effect against TA102 strain” by “When tested at the doses 50, 250 and 500 µg/plate, all extracts, mixed with 2-AA, showed a toxic effect on *S. typhimurium* TA102 cell viability.

Page 9, I replace the paragraph “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 towards a specific Salmonella strain. This mutagenic effect could be due to 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 (22)”. By “This effect may be ascribed to the formation of a complex between the mutagenic agent (2-AA) and extract components, which induces cell death at high concentration (22)”
Page 9 line 5, I replaced “For this reason, methanolic, aqueous and TOF extracts were tested at 5, 10 and 25 µg/plate, these doses did not influence TA102 strain viability (table 5). These extracts inhibit the mutagenicity of 2-AA by, respectively, 32.6%, 44.6% and 32.6% at respectively the lowest dose (5 µg/plate)”. By “That’s why we decreased extracts concentrations to 5, 10 and 25 µg/plate, as these doses did not influence bacterial viability (table 5). The tested extracts revealed an inhibitory effect against the mutagenicity of 2-AA by, respectively, 32.6%, 44.6% and 32.6% at respectively the lowest dose (5 µg/plate).”

Page 9, line 9, I replaced “these” by “A. salicina leaf”

Page 9, line 10, I delete “the”

Page 9, line 10, I replace “due” by “ascribed”

Page 9, line 16, I replaced “for providing” by “involved in”

Page 9, line 17, I added “in” between “and” and “preventing”

Page 10, line 5, I added “when extracts were combined with mutagene”

Page 10, line 9, I added “then”

Page 9, line 17, I replaced “The major differences between all the papers published by Bouhlel et al. (36) (44) and the present 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. “ by “The chemical analysis of leaf extracts from A. salicina,harvested from the south east of Tunisia (11) revealed important differences from those obtained from
A. salicina collected from the center of Tunisia (10) (29). These different may explain the different biological activities revealed by the two A. salicina ecotypifies.

I removed the last paragraph in the discussion, I replaced “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 had to use 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), is that it has been carried out by an enzymatic xanthine/xanthine oxidase system in the present papers, and by a nonenzymatic generating system (NBT/riboflavin) in the other one. 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:

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

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”. By “On the other hand, our study is in accordance with results by Mansour et al (12) as far as we confirmed the antigenotoxic effect of A. salicina extracts described by these authors, who is used a different
prokaryotic assay i.e. the SOS chromotest in the presence of *E. Coli* PQ37 described by Quillardet and Hoffman (30). However some differences arised from evaluating $O_2^\cdot$ scavenging effects when comparing antioxydant results of the present study and those reported by Ben mansour et al (12), Who revealed no scavenging effects against superoxyde anion. This could be explained by the different antioxydant assays used in each study. In fact Ben Mansour et al carried a nonenzymatic $O_2$ generating system to evaluated scavenging effects of A.salicina leaf extracts, Whereas, we used in the present study the enzymatic X/XOD superoxyde generating assay system to evaluate $O_2$ scavenger activity of the tested extract”.

I remove the conclusion “The experiments described above demonstrated the interaction between secondary metabolic composition of extracts with each radical and strain, 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 lines.” by “The results we obtained demonstrated a possible interaction between secondary metabolites in each extract with tested radicals, and with mutagenic assay strains. Polar extracts from (Tunisian) *Acacia salicina* leaves exhibited significant radical scavenging potential, as well as, antimicrobial and antimutagenic activities. This work paves the way for studying the effect of these medicinal plant components in the treatment of cancer cells by eventually inducing apoptotic death.induction of apoptosis in cancer cells lines.”

**Section Bacterial strains**

Page 13, line 2, I changed “carry” by “carries”

Page 13, line 7, I deleted “also”

Page 13 line 9, I changed “because it has an” by “by its”
Section *In vitro* antimicrobial activity

Page 13 line 2, I changed “and” by “as well as”

Page 13 line 4, I added “and”

Page 13 line 13, I corrected spelling of “suppresses”

- We tried to address the grammatical and typographical errors as asked by the reviewer.
- We corrected the English language.