Reviewer's report

Title: Analgesic principle from Curcuma aeruginosa

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Reviewer: Hellíada Chaves

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This study uses a plant extract from Curcuma aeruginosa, its fractions and an isolated compound to reduce the nociceptive response after acid acetic or formalin injection. This study is interesting in the field of nociception. However, there are several points need to be concerned.

- Major Compulsory Revisions

1. In the animal section of method, it is described that it was used both sex in the experiments of nociception. It is known that the inclusion of females is mandatory in clinical but not in preclinical studies. In fact, some studies have demonstrated the differences in the feeling of nociception and pain in animals and in humans depending on the sex or gender, respectively, beyond the knowledge that nociception is mediated through different mediators in male and female mice and probable involves sex hormones. Moreover, there are sex differences in many neurotransmitter systems.

2. It is necessary to review the methodology of tests. The method used to quantify the writhing is not the recommended one, which is a complete abdominal muscle contractions and hind paw extensions, besides the time of observation needs to be for 30 minutes. The authors observed for only 10 minutes the number of writhing, and the full writhing was counted as two half writhings taken as one full writhing. Also, the positive control group was diclofenac, which is not the most used drug for this purpose.

3. In the formalin-induced hind paw licking sections of method, why did the authors not proceed the experiments with SF. 1 to SF. 5? The authors may do it to complete the results data.

4. Also in the method section, why did the authors proceed the experiments with 200 mg/kg of VLC fractions in the acetic acid induced writhing and in the formalin-induced hind paw licking they used 100 mg/kg of VLC fractions?

5. The authors need to review the discussion about the formalin test. Studies have shown that non-steroidal anti-inflammatory drug has no effect on the first phase of the formalin test, even though the acetylsalicylic acid (ASA) and paracetamol were antinociceptive in both phases. It is contrary to the results obtained by this study, which reported a significant effect of aspirin just in the early phase of the formalin test.

6. Furthermore, the authors need the rewrite the first sentence of the second paragraph. The discussion is not correct since in the early phase of the formalin
induced licking is due to a direct effect on nociceptors, but it is not a test to conclude the involvement of the central nervous system, and it is neither correct to affirm that the late phase only indicates that a drug is a NSAIDs because studies has shown that not only NSAIDs have effect in this phase, but also analgesic opioids.

7. In a previous study (reference 4), it was observed that only the chloroform extract of C. aeruginosa suppressed the licking activity of the late phase in the formalin test in mice, but not the methanol extract of C. aeruginosa. In the present study, however, the authors show that the methanol extract exhibited inhibition of licking in both the early and the late phase. How can these differences can be justified?

8. The authors need to review the conclusion since it is not suitable to affirm that germacrone acts on the central nervous system.

9. The molecular mechanism of antinociception action of methanol extract from Curcuma aeruginosa, its fractions or the isolated compound germacrone is still unclear. This should be clarified by additional examination.

- Minor Essential Revisions

1. Title needs to be re-written. Since this work is yielded in experimental protocols, it is necessary to change the term "Analgesic principle" for "Antinociceptive principle". The changes are also necessary throughout the text.

2. In the section methods of the abstract, the authors used the expression "20, 40 mg/kg of 1", but the symbol 1 has not been described yet and it is not clear what it corresponds to. It is necessary to specify the term "of 1" before the authors cite it.

3. In the section methods of the abstract, the authors should include which groups were used for negative and positive control groups. It is also necessary to include the doses used for Fr 1-5 and Sf 1-5 in the nociceptive tests.

4. In the section methods and results of the abstract, the authors used the expression "compound 1". Sometimes the authors use the term "of 1", in other times the authors use the term "compound 1", and in other times the authors use the term "germacrone". It is necessary to standardize the terms.

5. In the section results of the abstract, the authors affirm that "Fr. 1 was found to have the most potent analgesic activity", but the data was not showed in results comparing the 5 fractions. The same was observed with Sf. 1-5. It is necessary to include data.

6. In the section results of the abstract, the unique data showed was related to the methanol extract of Curcuma aeruginosa and to the germacrone, but the authors did not do the correspondence between the percentages and the doses used for both the acid acetic and the formalin test. It is necessary to complete the results data.

7. In the section conclusion of the abstract, the authors affirm that "Germacrone showed a potent analgesic activity that acts on the central nervous system", but in the methods section, the authors contradict this sentence saying that the tests
chosen were used "to investigate a peripherally acting analgesic drug". In fact, neither the acid acetic nor the formalin test are methods to identify the involvement of the central nervous systems. To affirm that, the authors need to develop others experiments like hot plate or to administer substances via intra-thecal to test the supra-spinal mechanisms. It is suggested to change the sentences or to proceed the experiments.

8. In the key words of the abstract, the word "Zingiberaceae" was not cited in the text. The authors should insert the term in the abstract to justify its use as a key word. The same was observed for "Cyclic sesquiterpene".

9. In the background section, the authors comment the activities demonstrated for Curcuma aeruginosa in the literature, but there are activities that were put out of the text. It should be deserved attention for the papers below:


10. In the background section, the authors commented that germacrone, furanodiene, curcumenol, zedoarol, zedoarondiol, zedoalactone A, zedoalactone B, isocurcumenol, and isoaranciophene are the major chemical constituents isolated from the rhizomes of C.aeruginosa. Why the authors did not proceeded experiments with these compounds to certify that germacrone is in fact the principle antinociceptive compound?

11. From the method to results and discussion sections, the authors should standardize the presentation form of the symbols, putting or not space between the symbols and the numbers.

12. In the acetic acid induced writhing and formalin-induced hind paw licking sections of method, it is necessary to change the word "and" between the doses for "or" between them.

13. In the results and discussion section, the references 10 and 14 are not the best ones to discuss that "Acetic acid induced writhing is a useful method for investigating peripherally acting analgesic drug leads". Similarly it was observed with the references 11, 12 and 13 to discuss about the formalin test. It is necessary to change for a more appropriated ones.
14. In the results and discussion section, the tables 1 and 2 need to be cited early in the text.

15. It is already known that germacrone also exerted anti-inflammatory activity in carrageenan-induced hind paw edema in rats (Claeson et al., 1993). This information could be inserted in the discussion section.

16. There are not legends for the figures 1 and 2.

17. The format of the tables needs to be reviewed, they are out of the format recommended by the instructions for authors.

18. The references need to be also reviewed, they are out of the format recommended by the instructions for authors.

19. It is recommend to edit the manuscript by a native English speaker.

**Level of interest:** An article whose findings are important to those with closely related research interests

**Quality of written English:** Not suitable for publication unless extensively edited

**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.