Reviewer’s report

Title: Beneficial effect of Mentha suaveolens essential oil in the treatment of vaginal candidiasis assessed by real-time monitoring of infection

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Reviewer: Naho Maruyama

Reviewer’s report:

This manuscript evaluates that Mentha suaveolens essential oil has candidastatic and candidacidal activity in vitro, and also is effective against vaginal candidiasis model using newly investigated in vivo imaging technique. The topic is very interesting.

My comments include the following:

“Major Compulsory Revisions”

(1) Cytotoxicity assay towards immune cells

(a) Methods section (P9) described that 12 different concentrations was tested for each essential oils, but there were only 6 and 7 concentrations for L929 and Monomac6 in Figure 3. All data should be indicated.

(b) The author said “higer than MIC and MFC (P13, L12)”. I am not sure if these are the MIC and MFC of C.albicans, or those of Monomac6 and L929? If these are from Monomac6 and L929, their value should be mentioned. If these are from C.albicans, MFC of gLUC59 strain is 1.56 g/L, which is higher than 1000 mg/L, so the author can not say “higher than MFC”.

(c) C.albicans was incubated only for 2 hr in this experiment, meanwhile, it was incubated for 24-48 hr for other experiments such as MIC and time-kill test. From time-kill test (Fig 1), C.albicans looks alive after 2hr incubation at most of EOMS concentration. So, both C.albicans and immune cells might be able to survive for 2hr incubation at high EOMS concentration.

From these, I am afraid that it is difficult to think that the cytotoxicity of EOMS was low.

(2) Vaginal candidasis (P14 and Figure 5 and conclusion)

There is a lot of variation in the both photon emission and CFU data of saline (control) group on day 21. I am afraid that it might be difficult to evaluate the effect of essential oil on day 21. When the data was compared until day 15, the effects of TTO and EOMS were same level. Therefore, the phrase in discussion “Importantly, the EOMS activity in our model seems superior to that of TTO,” seems to be too emphasized.

The average and SD value were better to be added in figure.

CFU of EOMS was not significant on day 21 because p value (0.053) was more than 0.05.
p-value of TTO in photon emission on day 15 should be added in Figure, because it was significant as mentioned in result (P14).

I am not sure if non-parametric Mann-Whitney U-test was appropriate.

(3) Time-kill test (Discussion P16 L7-)
The author did not determine the time killing curve of TTO. So, they can not say “We determine the time killing curves, and so discovered that EOMS was apparently more effective than the more extesively investigated TTO”.

(4) Statistical analysis
The authors did not mention the result of t-test, even though it was written in methods part. The results of statistical analysis of time-kill assay and yeast and hyphae survival test should be described in results part and figures.

“Minor Essential Revisions”
(1) Jasmine oil (P6)
Jasmine oil is usually obtained by solvent extraction, not by steam distillation, and the extract is called “Jasmine absolute”. I want the author to confirm the extraction method. If jasmine oil is obtained by solvent extraction, other essential oil obtained by steam distillation was better as negative control.

(2) Methods - Hyphae survival (P8, L4)
Temperature for 4 hr incubation, and incubation condition (time, temperature) after essential oil treatment is better to be added, even though they were written in other part.

(3) Figures 1
Time points in x-axis should be accurate, not be equal interval. It is easy to understand when the name of Candida strain was added in Figure legend.

(4) Figure 2
(A&B): Results were better to be indicated as mean # SD (or SEM) like Figure 1 and 3.
(C): Photographs are not clear even though it might be caused by photographic sensitivity and/or my printer.

(5) Cytotoxicity assay (P13 and Figure 3)
The concentration of EOMS was written as µg/ml in figure 3, but as mg/L in P13. They should be unified.

(6) Figure 4 and 5
“Mint/Mint oil” and “Jasmine/Jasmine oil” should be “EOMS” and “JO”.

“Discretionary Revisions”
(1) It might be better to use not only TTO but fluconazole as positive control of in
vivo study, because methods were different from ref 10.

(2) Negative control might not be necessary except when the authors want to emphasize that Jasmine oil was non-effective.

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

**Quality of written English:** Acceptable

**Statistical review:** Yes, but I do not feel adequately qualified to assess the statistics.

**Declaration of competing interests:**

I declare that I have no competing interests.