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OPEN PEER REVIEW

Formulation and Physicochemical Evaluation of a 3% Cream Using Crude 70% Ethanolic Extract of *Morus alba* L. Leaves for Skin Hyperpigmentation

Shahab Edalatian Zakeri, Abbas Hadjiakhoondi, Saman Ahmad Nasrollahi, Mahdi Vazirian, Atefeh Naeimifar

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About this report

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1 Original submission

3 comments · 3 May 2021 – 4 May 2021

Referee 1 Reviewer

MINOR REVISION 3 May 2021

This is an interesting manuscript dealing with the formulation of a viscous oil/water emulsion for the treatment of skin hyperpigmentation based on a phenol-rich 70% ethanolic extract of Morus alba L. leaves. As far as I can see, all the pharmacognosy and pharmaceutics experiments were well performed, and the manuscript could be accepted after checking some minor points as shown in the attached modified PDF file. In particular, the following:

1. Line 126: milisiemes/cm
2. Line 135: Please check the first density value. Is it 0.68? Further, are the correct units g/ml instead of mg/ml? Check it.
3. Lines 166-188: Both paragraphs are not necessary here because this information was mentioned in the Introduction part.

Associate Editor

MINOR REVISION 3 May 2021

Dear Dr. Ahman Nasrollahi
The manuscript entitled "Formulation and physicochemical evaluation of a 3% cream using crude 70% ethanolic extract of Morus alba L. leaves for skin hyperpigmentation", which you submitted to the JJNPP, has been reviewed. The comments of the reviewer(s) are included at the bottom of this letter.
The reviewer(s) have recommended minor revisions to your manuscript. Therefore, I invite you to respond to the reviewer(s)' comments and revise your manuscript.
With regards
Neda Bavarsad

Editor-in-Chief

MINOR REVISION 4 May 2021

Dear Authors,

Please find the reviewers' comments attached; they have recommended minor revisions. Please revise your manuscript accordingly and submit your revised manuscript with a point-by-point answer to the comments for further processing.

We are looking forward to receiving your revised manuscript.

Kind regards,

Editor-in-Chief

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Revision 1

AUTHORS' RESPONSE TO REVIEWERS

Authors' reply to reviewers

The next pages contain the authors' point-by-point reply to the comments of this revision round, as submitted with the revised manuscript. It is published as the authors wrote it.

Dear Prof. Zahra Ramezani
Editor-in-Chief, Jundishapur Journal of Natural Pharmaceutical Products

I would like to thank you all for the very careful review of our manuscript titled: **“Formulation and physicochemical evaluation of a 3% cream using crude 70% ethanolic extract of Morus alba L. leaves for skin hyperpigmentation”**

Following kindly you may find our responses to the reviewer’s comments. Also, the revised manuscript was corrected accordingly and the changes were highlighted.

Sincerely,

COMMENTS

Reviewer 1:

1. The scale bar or magnification should be provided for Figure 1.

The figure 1 legend was changed to "*Oil droplets in the aqueous phase (40X magnification)*"

2. The Figure 2 should be presented with proper caption containing solvent type that the standard curve plotted in and number of replicate of samples. Axis title also should be added for X-axis.

In the revised manuscript, the figure 2 caption changed to "*Calibration curve of different concentration of galic acid in ethanol 70%, n=3*"

Galic acid concentration (µg/ml) was added to X-axis.

3. The author should explain how “microbial assessment” study was performed in method section.

The fowlloing sentences was added to the revised manuscript in method section:

Microbial assessments

10-gram of cream was mixed with 3 g of sterile polysorbate 80; then, 24g of 10% tween 60 water solution was added to the TSB medium to diagnose the bacteria (mixture 1). Plates with 25-250 colonies were calculated, and the average number of CFU/g was counted. Two dilutions (10^{-1}, 10^{-2}) with the TSB culture medium were prepared, and 1-2 mL of pre-prepared dilution was added to 2 sterile plates containing 10-20 mL of TSA culture medium and incubated for 24 - 48 hours at 30 - 35°C (Pure plate). One mL of 0.1 dilutions for fungal counting allowed the agar to solidify at 25±2 °C, then for 5 - 7 days incubated at 20 - 25°C. Colonies were counted after the incubation; all preparations were carried out in triplicate. All microbial counts were carried out via pour plate and incubated for 3 days, and then counting was performed. Counting was approved in plates containing fewer than 100 colonies for microbial count (TAMC) and in those comprising less than 10 colonies for fungal count (TYMC).

- *Salmonella count*

Colonies were grown in Rappaport Vassiliadis Salmonella Enrichment broth medium and counted after 18- to 48-hour incubation periods at 30-35°C.

- *Enterobacteriaceae count*

Colonies were counted after 18-24 hours of incubation at 30-35°C.

- *Candida albicans count*

10 ml of mixture A was added to 100 ml SDA medium culture and incubated at 30-35°C for 3-5 days. After growing and colonies, it was incubated for 24-48 hours.

- *Escherichia coli count*

Colonies were grown in MacConkey broth medium and counted after 18 to 72 hour incubation periods at 30-35°C.

- *Pseudomonas aeruginosa count*

Mixture 1 was grown in Cetrimide Agar and incubated at 30-35°C for 18-72 hours.

- *Staphylococcus aurous count*

After having any S.aurous growth in mixture 1, it was cultured in Mannitol Salt Agar and incubated at 30-35°C for 18-72 hours, and the colonies were counted.

Preservative effectiveness test

A standardized inoculum containing three bacteria (P. aeruginosa, E. coli, and S. aureus), a yeast (C. albicans) and a mold (Aspergillus niger) was mixed with the liquid product. The mixture comprising 0.05% of polysorbate 80 was added to adequate sterile buffer phosphate (pH: 7.2) to gain a count of about 1 $\times 10^8$ CFU per mL. The absorption of the inoculum was seen at 580 nm. 10 gram of preparation was added to 90 ml buffer phosphate (Mixture 2) and 101 and 102 dilutions of the mixture B was prepared. Then, 1 mL of 10^1 and 10^2 dilutions was added to a sterile plate comprising 15 - 20 mL of the TSA medium, which cooled to 45°C, and incubated for 24-48 hours at 30-35°C. The fungal process was alike to the above excluding the SDA culture with 10^1 dilution was incubated for 5-7 days at 20-25°C. Positive and negative controls were used during the test. Microbiological examinations were performed for preparations at 25ºC after 14 and 28 days of the formulation.

4. In method section the author should describe briefly about protocols used for pH, density and viscosity measurement.

Folowing sentences was added to the revised manuscript in method section:

Determination of pH

The digital pH meter (Metrohm 827- Swiss) was used to measure the pH of the preparation. Examinations were repeated in triplicate. The pH meter was calibrated using standard buffer solutions (pH 4 and 7) before use.

Density

The density of a substance is its mass per unit volume. The exact volume of pycnometer was measured by filling it completely with water and the sides were lightly tapped to eliminate the air bubbles. The container was weighted full of a cream, and simply the density was examined.

$$p = mV$$

p = density, m = mass, V = volume

Viscosity

The cream viscosity was assessed by a Polyvisc Viscometer (Brookfield-USA) using a spindle R6 at 100 rpm. Viscosity measurements were carried out at room temperature (25±2°C). *All measurements were performed in triplicate.*

Viscosity in centipoises (cps) = Dial reading × factor

5. In Table 1: I suggest to make the statistical comparisons between different times with initial and among 1st and 3rd months (ANOVA). Number of sample replicate and relative standard deviations should also be added.

The samples was performed in triplicate and mean±SD was added in table 1 in the revised document.

The following sentences was added to method section in the manuscript:
Statistical analysis
Values in all experiments are represented as mean±SD of at least 3 independent experiments. Statistical analysis was performed by repeated measurement ANOVA test. The significant level was also set as $P < 0.05$.

Also in result section of revised document, the below paragraph was added:
There were no significant changes within stability time in assay, pH, density and viscosity (P-value of all following physicochemical characteristics was greater than 0.05).

6. In Table 1: what temperature the viscosity measured in?
Viscosity determinations were carried out at room temperature (25±2°C). Also this item was added to the method section.

7. Line 166: “Hyperpigmentation disorders occur especially in individuals with darker skin tones and those who are more exposed to the sun. It has also been linked to genetics and hormonal fluctuations in women” please provide proper references.
This paragraph (lines 166-188) was deleted as the reviewer 2 requested.

8. Lines 182-188: this paragraph may be presented in introduction section instead of discussion.
This paragraph (lines 166-188) was deleted as the reviewer 2 requested.

9. In author contribution section: are there any clinical data provided in this study?
The clinical study is underway and will be reported separately.

Reviewer 2:
1. Line 126: milisiemes/cm
This unit was added to the text.

2. Line 135: Please check the first density value, is it 0.68? Further, are the right units g/ml instead of mg/ml. Check it.
The initial density was 0.98 g/cm^3 but in the manuscript it was written incorrectly. The right unit is (g/cm^3). Thus, the right number and unit was added in the revised manuscript.

3. Lines 166-188: Both paragraphs are not necessary here because this information was mentioned in the Introduction part.
In the revised document, both paragraphs (lines 166-188) was deleted.

2 Revision 1

2 comments · 15 May 2021 – 17 May 2021

Associate Editor

ACCEPT 15 May 2021

Dear Dr. Nasrollahi
I am pleased to inform you that JJNPP would like to publish your manuscript.

Editor-in-Chief

ACCEPT 17 May 2021

Dear Authors,

I am glad to inform you that your manuscript has been accepted for publication in JJNPP. Your manuscript will be sent to the publisher for further processing.
We thank you for submitting your research results and look forward to receiving your future outstanding research results.

Kind regards,
JJNPP EIC