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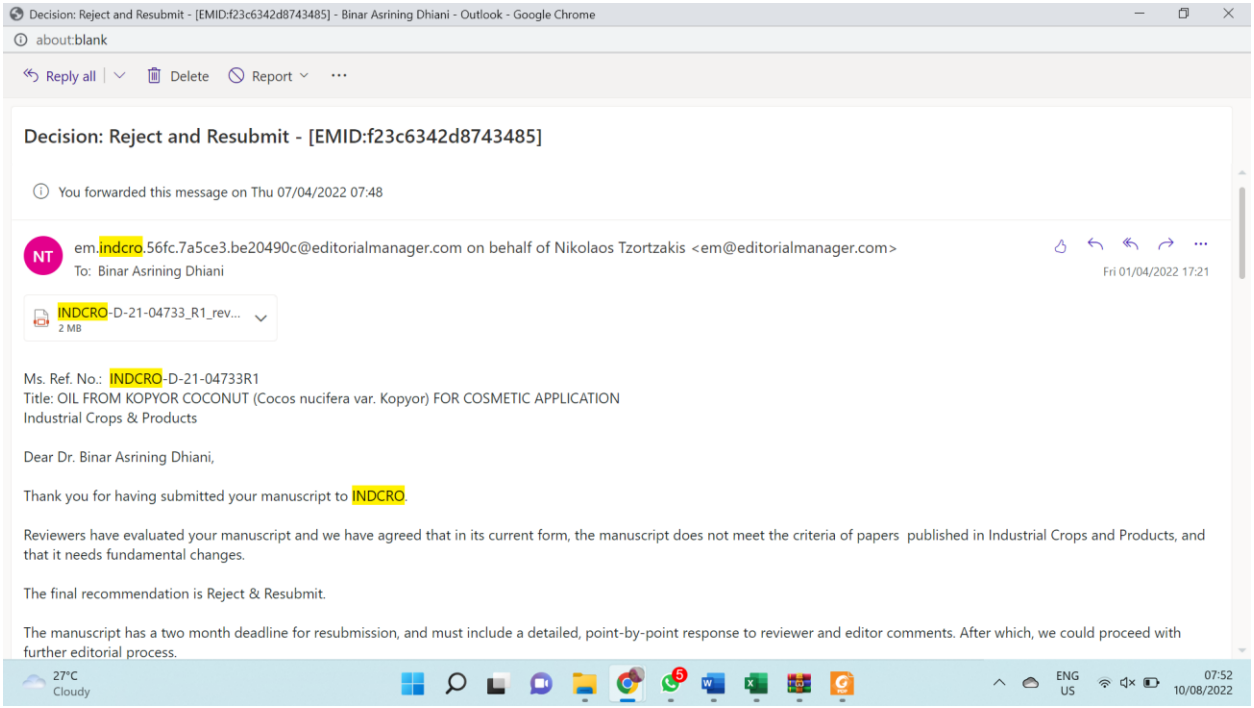

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Title: OIL FROM KOPYOR COCONUT (Cocos nucifera var. Kopyor) FOR COSMETIC APPLICATION
Industrial Crops & Products

Dear Dr. Binar Asrining Dhiani,

Thank you for having submitted your manuscript to INDCRO.

Reviewers have evaluated your manuscript and we have agreed that in its current form, the manuscript does not meet the criteria of papers published in Industrial Crops and Products, and that it needs fundamental changes.

The final recommendation is Reject & Resubmit.

The manuscript has a two month deadline for resubmission, and must include a detailed, point-by-point response to reviewer and editor comments. After which, we could proceed with further editorial process.

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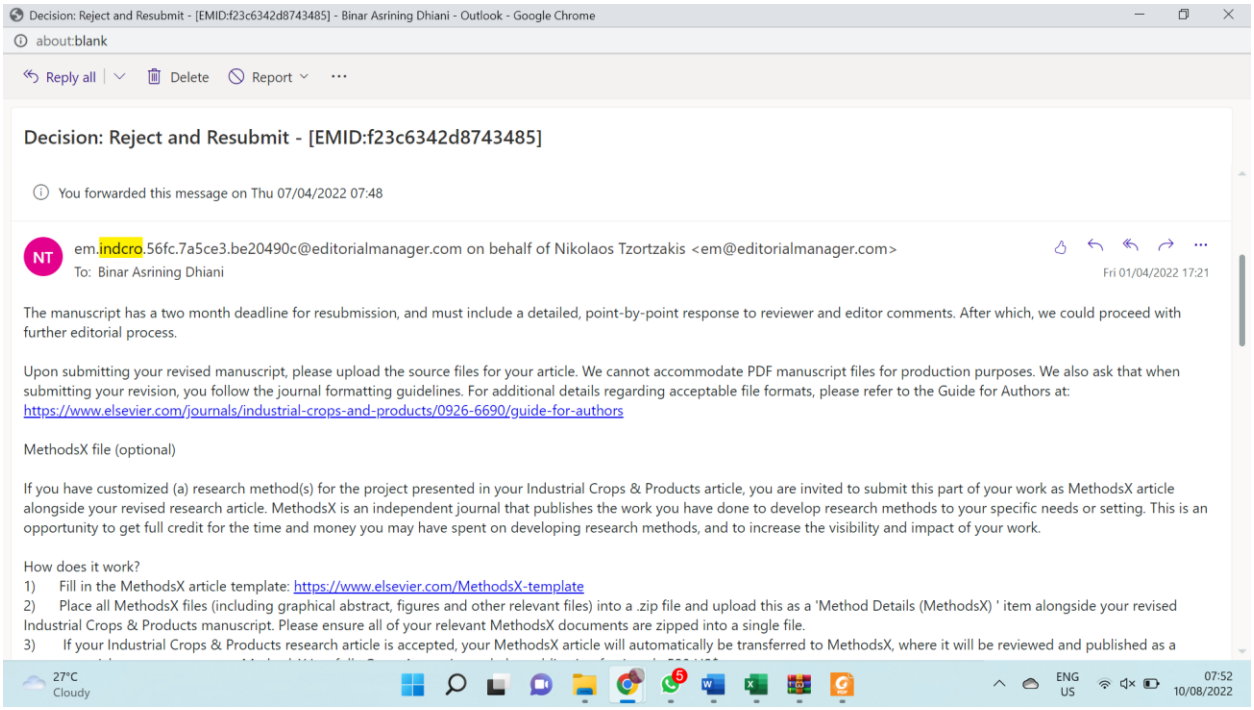

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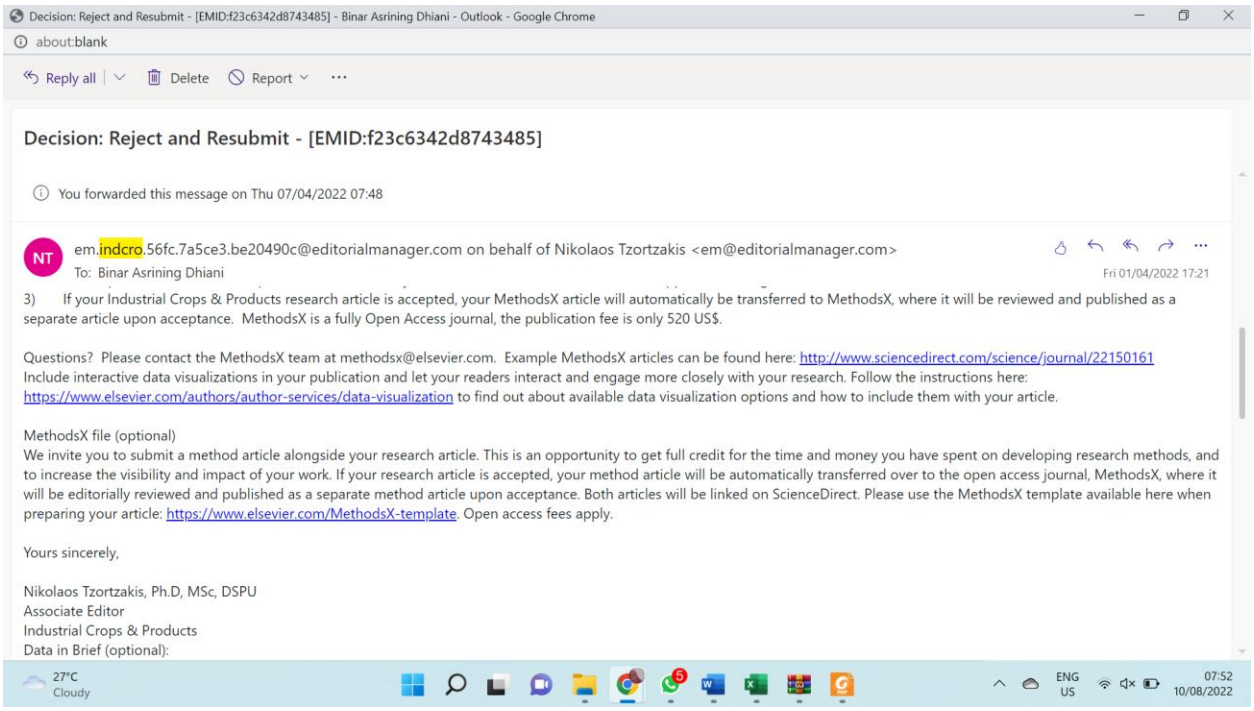
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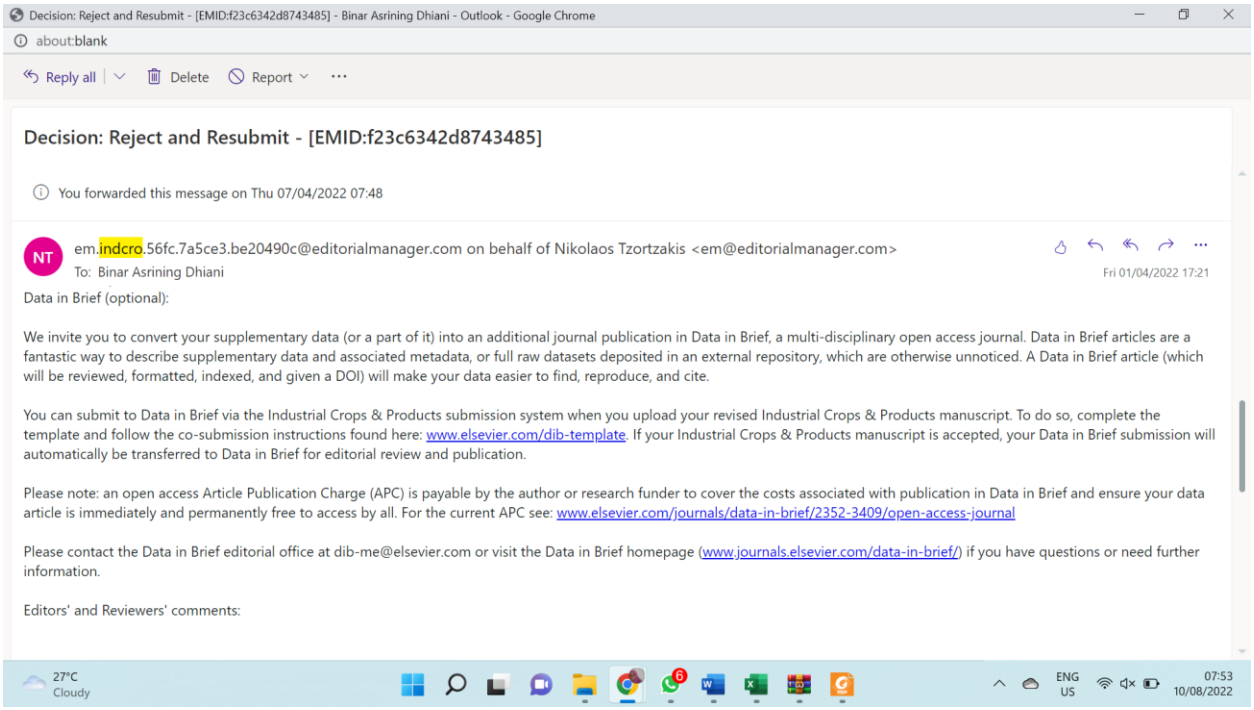
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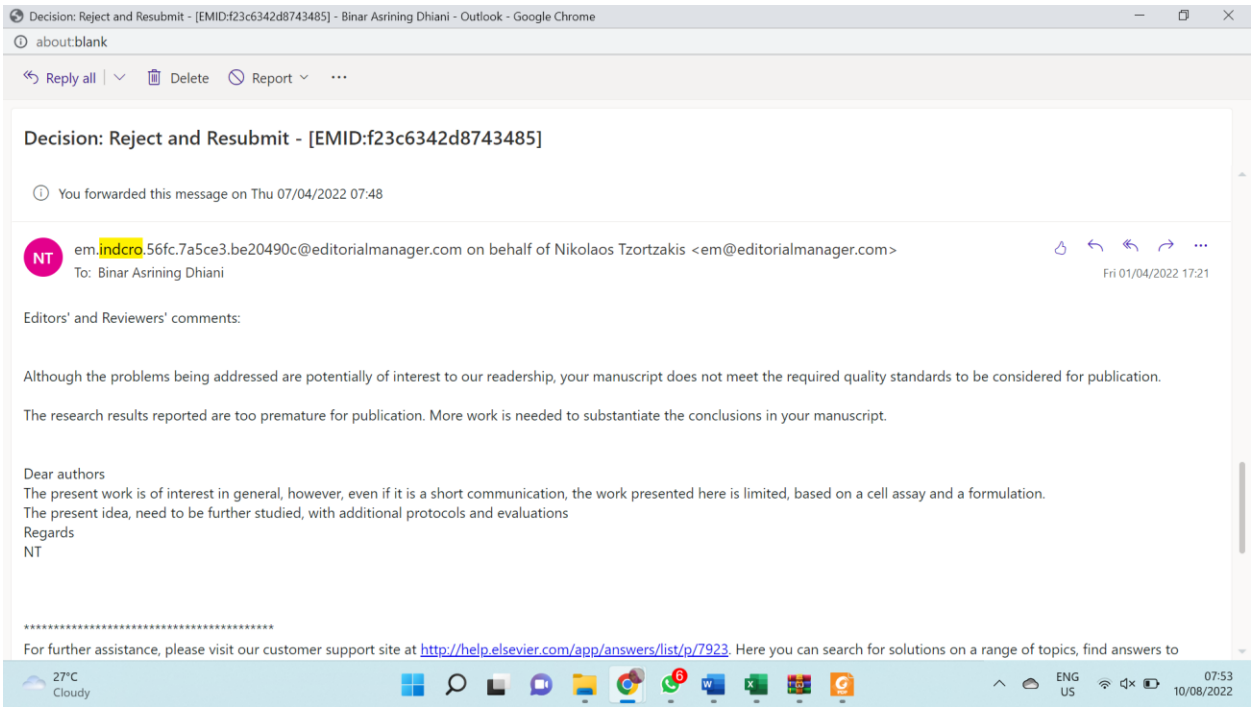
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Although the problems being addressed are potentially of interest to our readership, your manuscript does not meet the required quality standards to be considered for publication.
The research results reported are too premature for publication. More work is needed to substantiate the conclusions in your manuscript.
Dear authors
The present work is of interest in general, however, even if it is a short communication, the work presented here is limited, based on a cell assay and a formulation.
The present idea, need to be further studied, with additional protocols and evaluations
Regards
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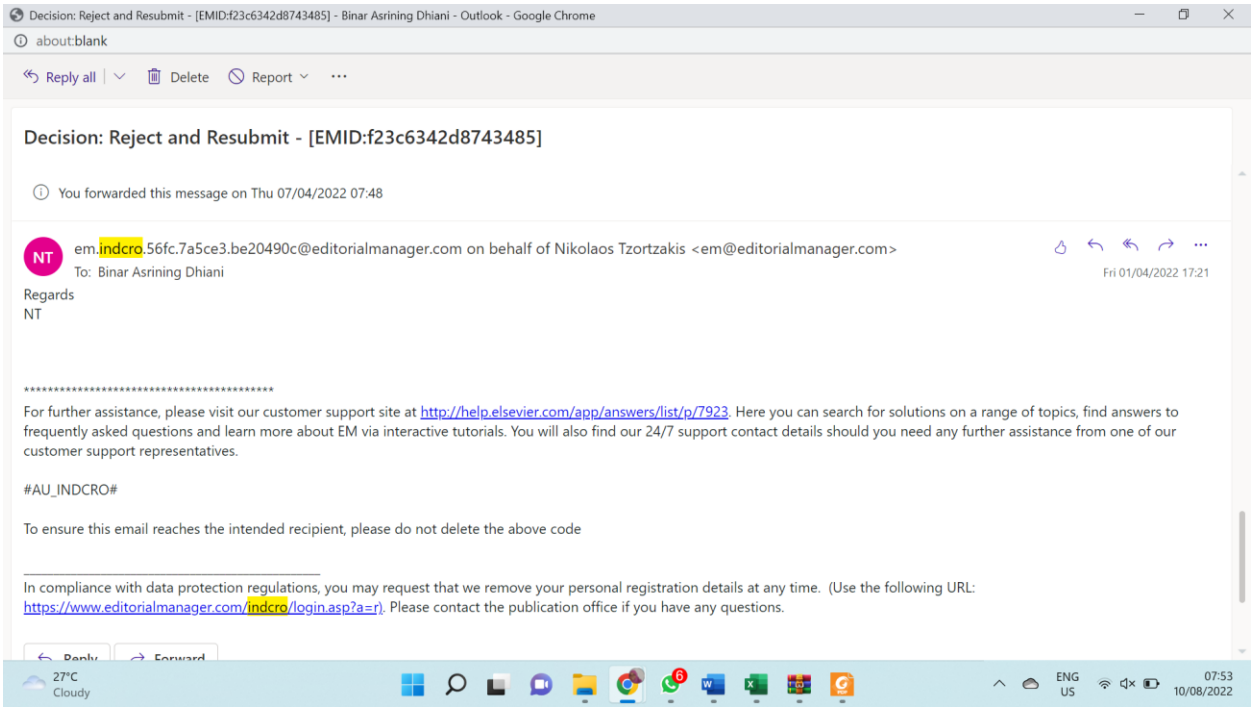
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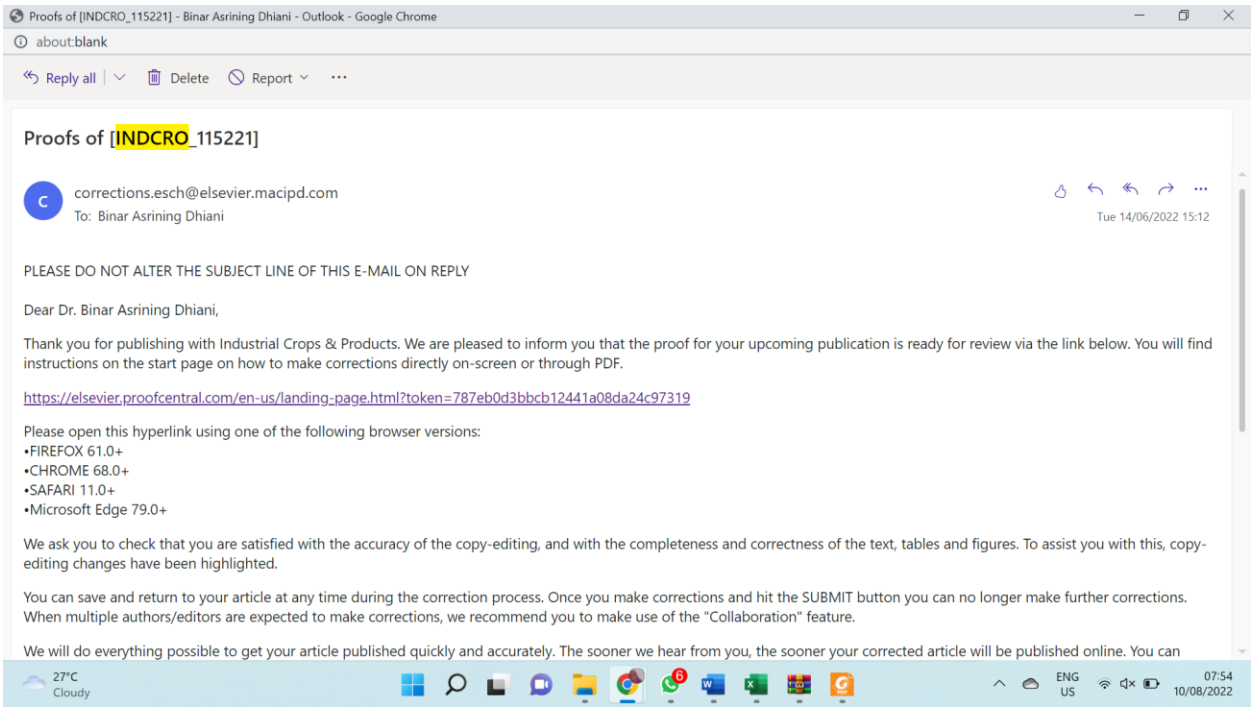
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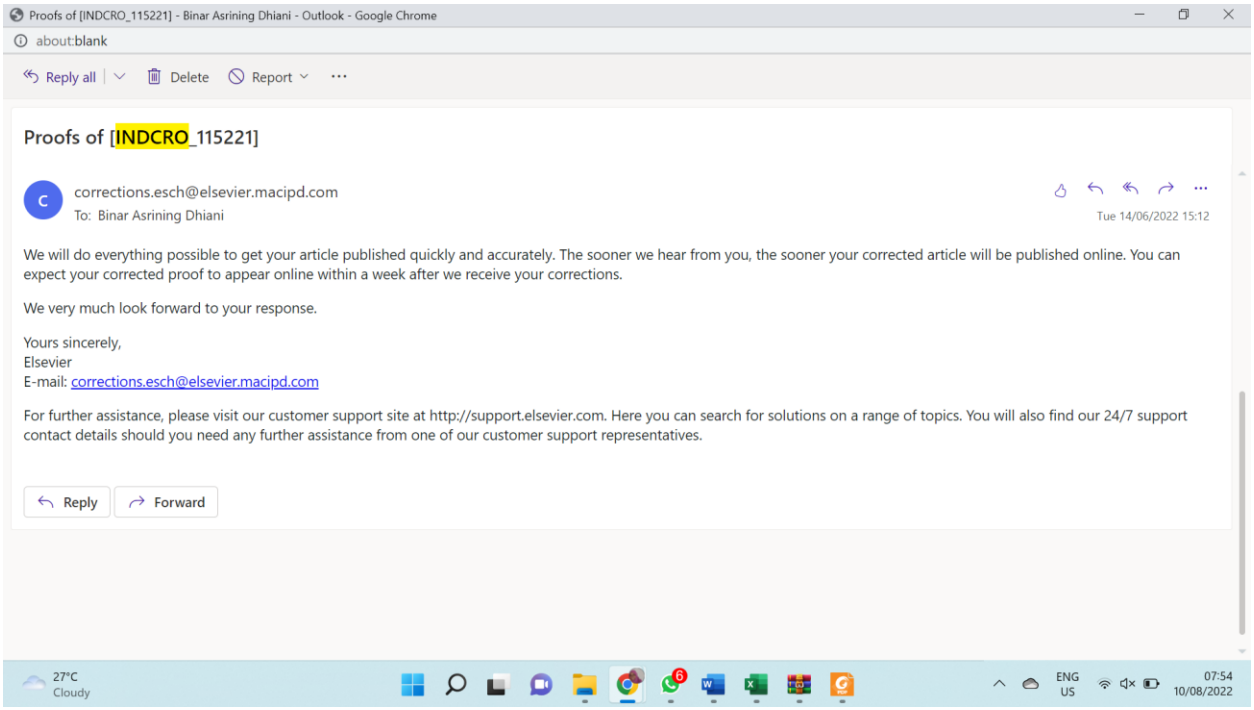
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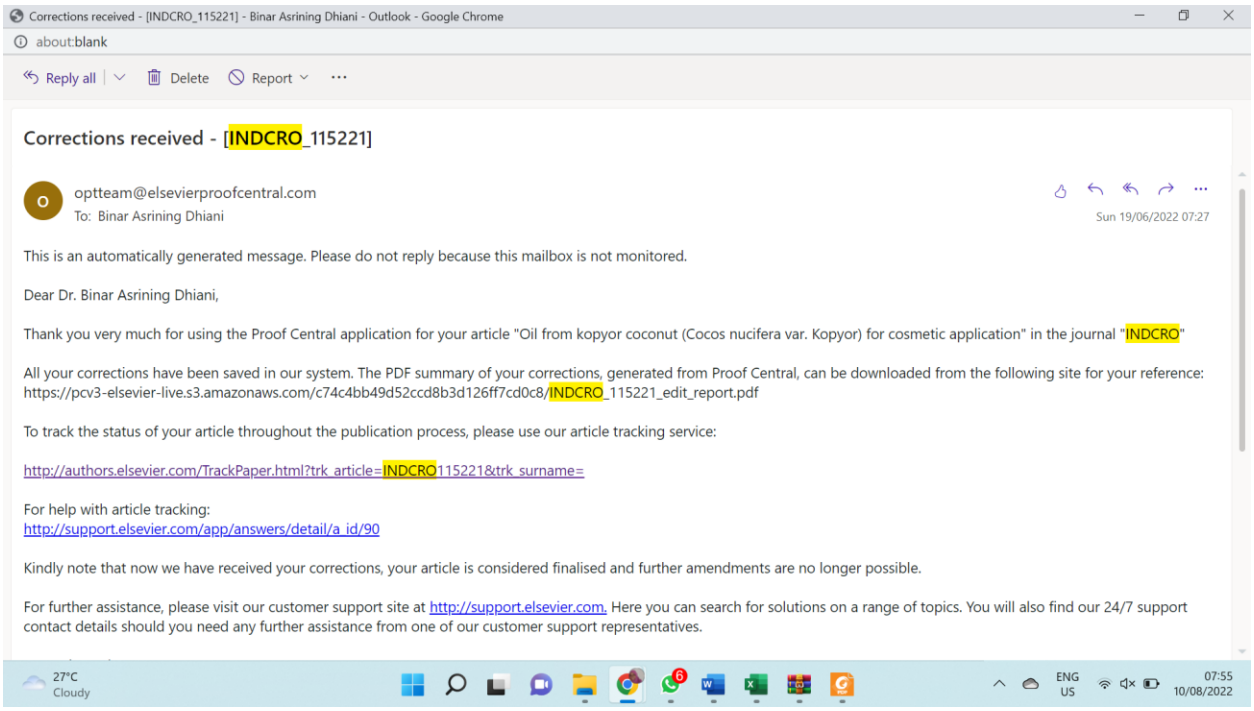
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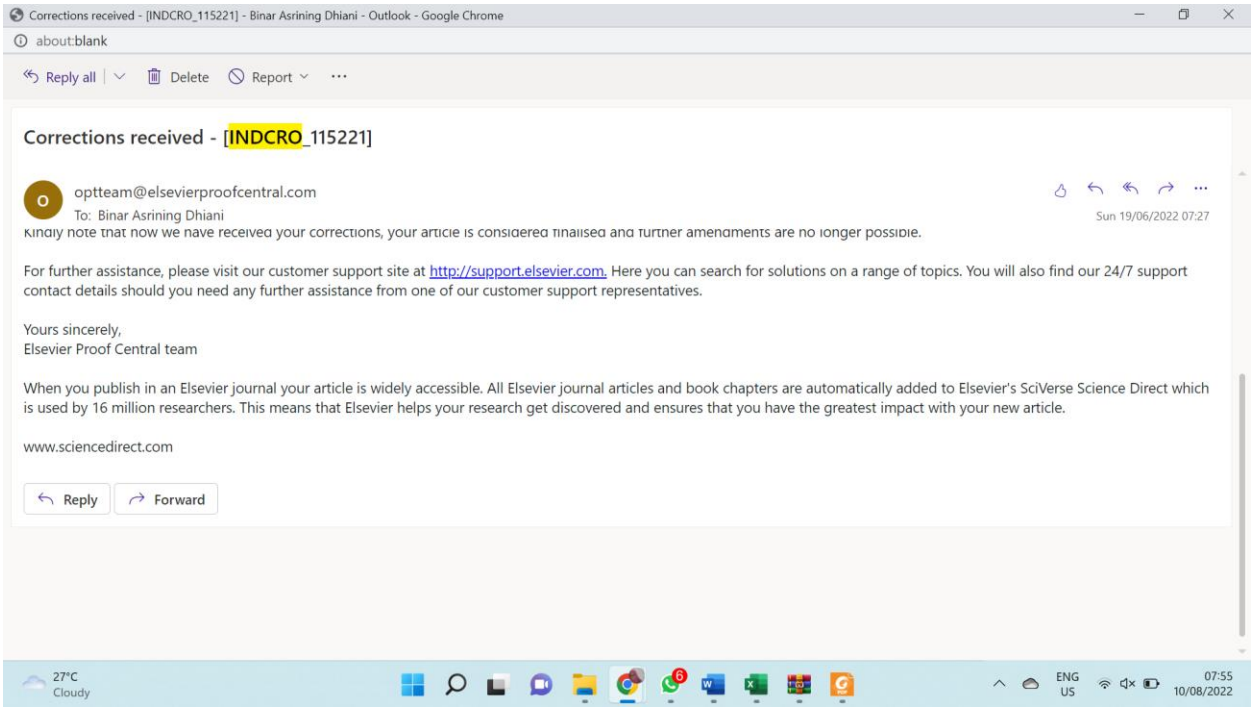
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Response to Reviewers

Dear Dr. Wiem Thielemans,

Thanks for your time to assess our submitted article.

Based on your request, we have uploaded the revised manuscript in the online submission system along with other required files separately, i.e., this letter, Highlights, Source files (for two figures in JPG/TIF files), and Supplementary Files.

Our article has been revised following author guidelines and your request as follows:

1. 'Please convert the line numbers to continuous line numbers.'

   Action: We have already converted the line numbers to continuous line numbers, as seen in the manuscript.

2. We added footnotes for the abbreviation required.
3. We have also corrected minor errors in spelling and grammar that we have found later.

We are looking forward to our article can be proceeded to the peer-reviewed process and published in Industrial Crops and Products.

Thank you.

Sincerely yours,

Dr. Binar Asrining Dhiani

Author response to editor and reviewers' comments (INDCRO-D-2104733-R1)

Title: Oil from Kopyor Coconut (Cocos nucifera var Kopyor) for Cosmetic Application

EDITOR

Comment:

Although the problems being addressed are potentially of interest to our readership, your manuscript does not meet the required quality standards to be considered for publication.

The research results reported are too premature for publication. More work is needed to substantiate the conclusions in your manuscript.

The present work is of interest in general, however, even if it is a short communication, the work presented here is limited, based on a cell assay and a formulation.
The present idea, need to be further studied, with additional protocols and evaluations

Response:

Thank you for your comments.
In order to meet the required quality standard for publication in this journal and to provide more evidence to support the conclusion of our manuscripts, we performed several experiments and additional data are provided in the revised manuscript. The data are mainly from initial safety assessment and alpha tocopherol content identification of KC oil and coconut oil as required. We conducted a primary irritation test in rabbits for the safety assessment. Identification and quantification of alpha tocopherol content were performed by HPLC method.
Revised version of the manuscript based on the reviewer's comments was also sent. The revised part in the new manuscript is shown in red letter. Our response point-by-point to the reviewer's comments can be found on this page.

REVIEWER #1

General Comment:

In the manuscript (INDCRO-D-2104733-R1) authors describe the development of Kopyor Coconut oil incorporated skin lotion. The estrogenic potential of tocopherol in the Kopyor oil has been suggested as the beneficial part. The efforts of the authors to develop a lotion are appreciable however the manuscript has the following de merits.

General Response:

Thank you for your appreciation and valuable comments. All comments are kindly appreciated and we respond to your comments in detail for each following comment.

***Comment*:**

1. Cell culture methodology is not clearly written and there are flaws in the design. It is not clear how long the cells were exposed to the defined concentrations in serum free media. In order to assess the interference of oil in the MCF cells proliferation, at least 48hr incubation is needed. Also the addition of

above 10% oil concentrations may not be physiologically relevant in monolayer cultures and in aqueous medium it would not get incorporated.

***Response*:**

1. We revised the cell culture methodology part to improve the clarity
2. It was mentioned in the old manuscript (page 5 line 109) that the cells were exposed to KC oil in three days (or 72 hours). We do agree that oil-based samples are needed more time to incorporate into the cells. This longer time incubation is also applied in the estrogenic activity study using MCF-7 cell-based assay. Thus, we performed 3 days of incubation of the tested compound in the cells before MTT addition as already written.
3. We prepare the KC oil in the defined series concentration in the DMSO as a solvent. DMSO can dissolve both oil and aqueous compounds. Hence, KC oil in DMSO allows the KC oil to dissolve in the aqueous media and incorporate into the cells. We were convinced with our sample preparation as we could see dose-dependent in our MCF-7 cell assay result.

Comment:

2. This cell based study is only an indirect assumption for estrogenic activity. No direct results for the estrogenic activity of the oil have been given. Also in the 5% oil used in the lotion, it is not clear there will be sufficient alpha tocopherol to provide estrogenic effect.

Response:

1. Yes, we do realize that the MCF-7 cell-based assay is an indirect method for estrogenic activity determination. Thus, we write this manuscript in a short communication to provide initial evidence that the KC oil has promising estrogenic activity.
   We also attempted to predict the estrogenic activity of KC oil based on the computational analysis as it was written in the old version manuscript on page 8. The molecular docking analysis of the alpha-tocopherol and ER-alpha showed that they do establish an interaction. This computational study revealed the rationale of how KC oil could increase estrogenic activity. These two methods provide sufficient evidence in the initial phase to proceed on to further extensive studies.
   We do need a thorough investigation to study the estrogenic activity in animal models such as ovariectomized rats or cynomolgus monkey to provide stronger evidence. We would also proceed to a limited clinical trial to prove the estrogenic activity of KC oil products. However, it would take a longer time and will not serve our purpose to publish it as short communication and quickly share the potential application of KC oil products.
2. We performed the identification and quantification of alfa tocopherol contained in KC oil and made a comparison with virgin coconut oil by HPLC. The KC oil contain alpha tocopherol higher than coconut oil; 1.47 g/L compared to 0.48 g/L. Based on the article reported by Lee et al., 2009, 100uM alfa tocopherol (4.31 mg/100mL) increased the MCF-7 cell growth up to 800%. The 5% KC oil used in this study contains alfa tocopherol 7.35 mg/100mL, almost doubled number. Even though, the comparison is made based on the cell-based assay, it provides evidence that 5% KC oil is sufficient to achieve the estrogenic effect.

Comment:
3. Either with the support of literature or comparative study from their experimental set up advantage of KOPYOR oil is not discussed
Response:
We made correction and added discussion as requested on page 13 line 271

Comment:
4. In the lotion prepared, there are other components present and the possible advantage of incorporation of Kopyor oil over other ingredients has not been discussed.
Response:
We made correction and added the discussion as requested on page 13 line 274

Comment:
5. Finally there are mistakes like usage DMEM 'medium" here the word medium is a repetition and have to be removed. Also check for typos and grammatical errors.
Response:
We made correction as requested on page 4 line 84, page 5 line 105

REVIEWER #3
Comment:
(page 2 line 34) Why is centrifugation method reported in '%"?
Response:
We write the percentage (5-100%) referred to the percentage of KC oil used in the estrogenic activity determination, not for the centrifugation force. We rewrite the sentences to avoid misinterpretation.

Comment:
(page 2 line 36) Write down the full form of 'EC50'
Response:
We made correction as requested on page 1 line 36

Comment:
(page 2 line 39) It is evident from abstract that there are three variables i.e., %KC, %stearic acid and %TEA. But only two variables are mentioned in design optimization. Why??
Response:
The percentage of KC oil was defined based on the estrogenic activity. We used 5; 10;20; 40; 80; and 100% KC oil in DMSO for estrogenic activity determination. It showed that 5% KC oil was the lowest concentration that is needed to increase the MCF-7 cell viability, which indicated its estrogenic activity. Thus, we applied 5% KC oil as a fixed parameter in the lotion formula design. The formula design optimization was then performed on only for two variables i.e., % stearic acid and %TEA.

Comment:

(page 4 line 91) Mention the name of reagents and source of procurement.
Response:
We added the information as requested on page 4 line 92-93

Comment:
(page 4 line 96) Provide configuration of food blender
Response:
We added the information as requested on page 4 line 97

Comment:
It will be better if you represent the speed of centrifugation in 'g'.
Response:
We changed the unit for centrifugation force as requested page 4 line 100

Comment:
Double first bracket is not allowed. Change it.
Response:
We changed the formula writing as requested page 5 line 117

Comment:
(page 9 line 184) Why is it 'probable'? Why was any method not employed to make a comparative study between KC and coconut oil?
Response:
We performed the primary irritation test in rabbits for determining the primary irritation score and index of KC oil, coconut oil, and KC oil lotion. The method was written on page 7 line 145-161. The result exhibited that the KC oil and lotion containing KC oil was not showed any erythema or very slight erythema comparable to coconut oil which did not show any erythema.
We made the correction and added this in the result and discussion section (page 10 line 213-220)

Comment:
(page 9 line 191) Mention the role of other ingredients (apart from KC oil, stearic acid and TEA) in the lotion.
Response:
We added the information as requested in Table 4.

Comment:
(page 10 line 199) Mention any standard body's reference for pH.
Response:
We added the reference as requested (page 12 line 234)

Comment:

(page 10 line 207) Provide valid justification of your conclusions on lotion stability up to 1 year based on the results of 5 h of centrifugation. This is too extrapolative and needs cientific support.

Response:

We did also the stability test experiment to investigate the lotion stability using cycling test. The test was performed in 6 cycles. The KC oil lotion was incubated at 4°C for 24 hours and then moved to the incubator set at 40°C for 24 hours for one cycle. This process was repeated for 6 cycles, and observed the physical appearance of lotion. It revealed that the lotion was stable and there was no characteristic changes before and after the cycling test.

We added this information on page 12 line 250.

Comment:

An animal model study for topical application must be conducted to conclude this.

Response:

We conducted a simple skin analysis using Skin Analyzer EH-900U tools to show the percentage of elasticity, moisture, and sebum after KC oil application for 3 weeks (21 days). It exhibited that KC oil lotion was able to maintain skin normal elasticity, high water content, and balance the oil secretion (please see Table 6, page 13 line 251). Thus, the data were able to provide stronger evidence of the promising application of KC oil for a cosmetic product.