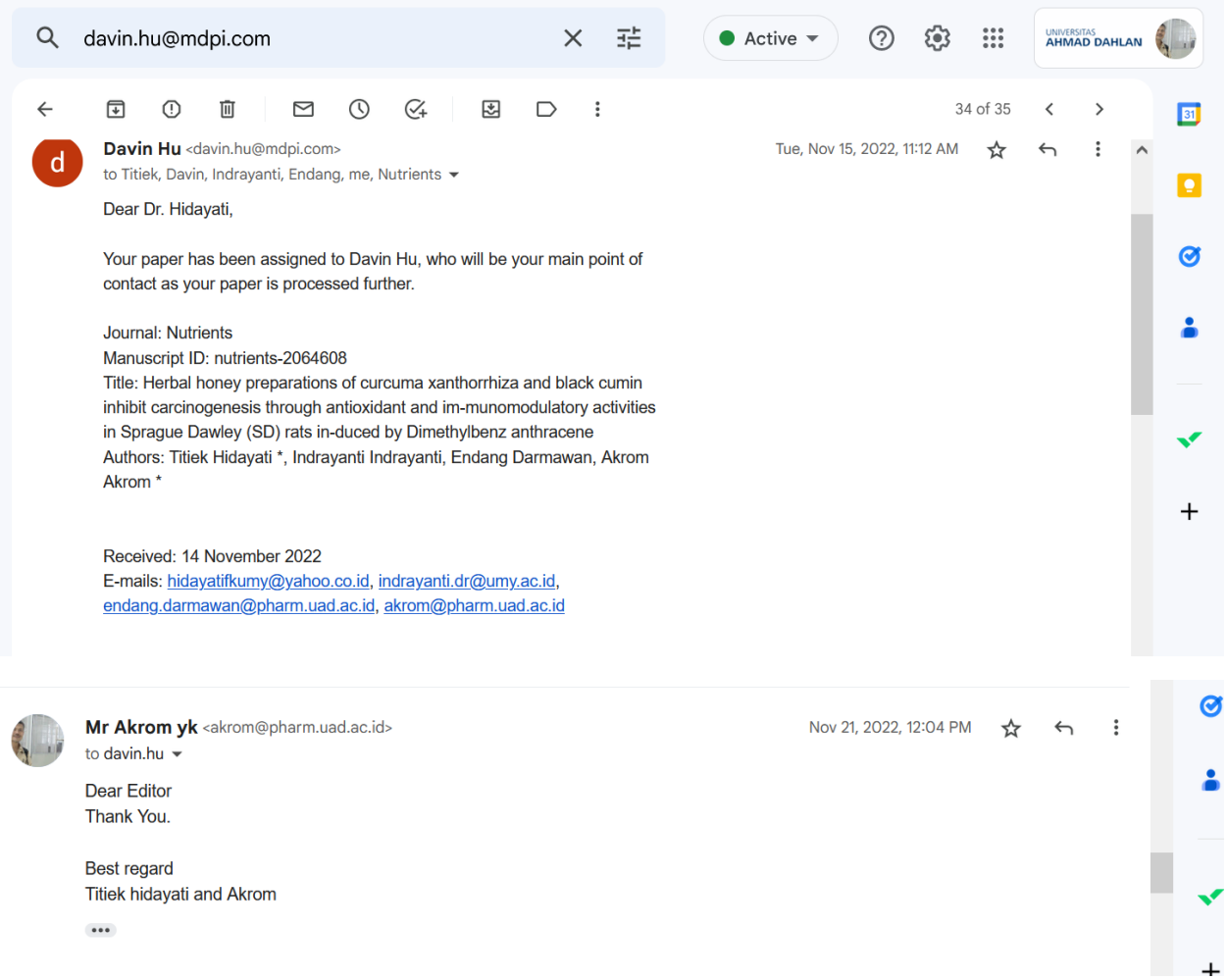
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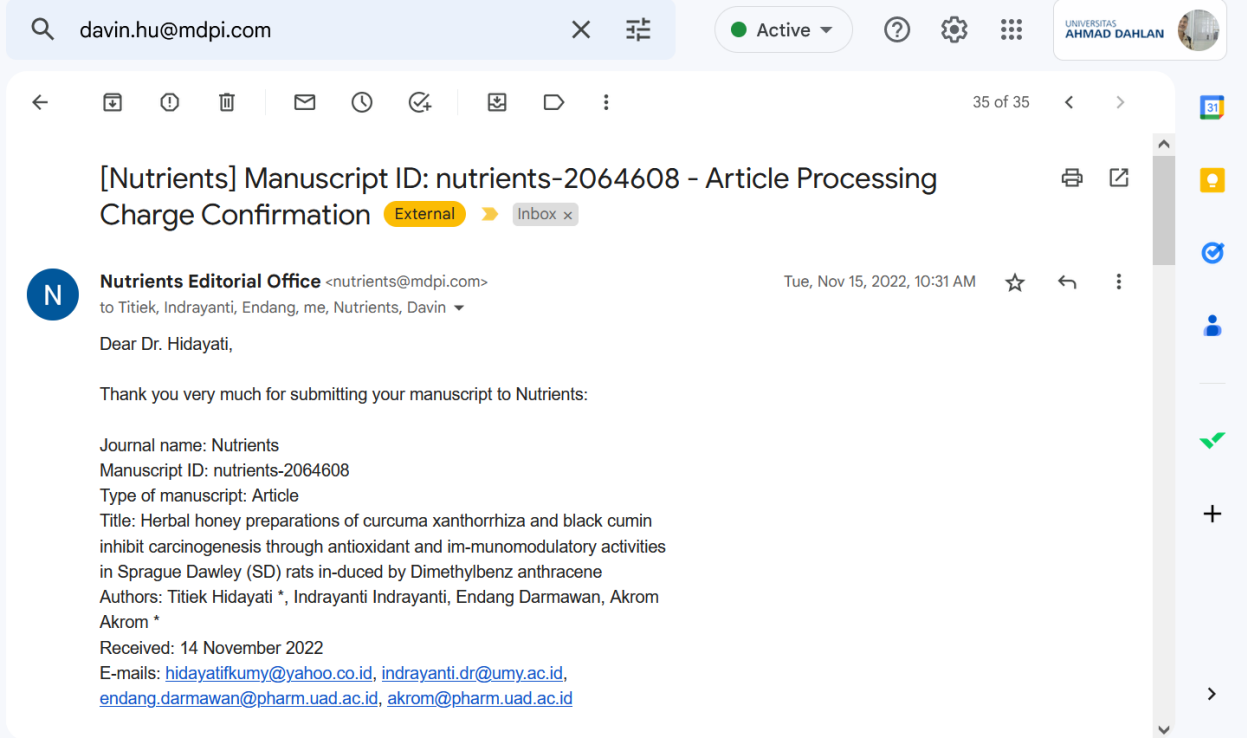
34 of 35
Davin Hu <davin.hu@mdpi.com>
to Titiek, Davin, Indrayanti, Endang, me, Nutrients
Tue, Nov 15, 2022, 11:12 AM
Dear Dr. Hidayati,
Your paper has been assigned to Davin Hu, who will be your main point of contact as your paper is processed further.
Journal: Nutrients
Manuscript ID: nutrients-2064608
Title: Herbal honey preparations of curcuma xanthorrhiza and black cumin inhibit carcinogenesis through antioxidant and im-munomodulatory activities in Sprague Dawley (SD) rats in-duced by Dimethylbenz anthracene
Authors: Titiek Hidayati *, Indrayanti Indrayanti, Endang Darmawan, Akrom Akrom *
Received: 14 November 2022
E-mails: hidayatifkumy@yahoo.co.id, indrayanti.dr@umy.ac.id, endang.darmawan@pharm.uad.ac.id, akrom@pharm.uad.ac.id

Mr Akrom yk <akrom@pharm.uad.ac.id>
to davin.hu
Nov 21, 2022, 12:04 PM
Dear Editor
Thank You.
Best regard
Titiek hidayati and Akrom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[Nutrients] Manuscript ID: nutrients-2064608 - Article Processing Charge Confirmation
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Inbox ×
Nutrients Editorial Office <nutrients@mdpi.com>
to Titiek, Indrayanti, Endang, me, Nutrients, Davin
Tue, Nov 15, 2022, 10:31 AM
Dear Dr. Hidayati,
Thank you very much for submitting your manuscript to Nutrients:
Journal name: Nutrients
Manuscript ID: nutrients-2064608
Type of manuscript: Article
Title: Herbal honey preparations of curcuma xanthorrhiza and black cumin inhibit carcinogenesis through antioxidant and im-munomodulatory activities in Sprague Dawley (SD) rats in-duced by Dimethylbenz anthracene
Authors: Titiek Hidayati *, Indrayanti Indrayanti, Endang Darmawan, Akrom Akrom *
Received: 14 November 2022
E-mails: hidayatifkumy@yahoo.co.id, indrayanti.dr@umy.ac.id, endang.darmawan@pharm.uad.ac.id, akrom@pharm.uad.ac.id

We confirm that, if accepted for publication, the following Article Processing Charges (APC), 2600 CHF, will apply to your article:

Journal APC: 2600 CHF
Total APC: 2600 CHF

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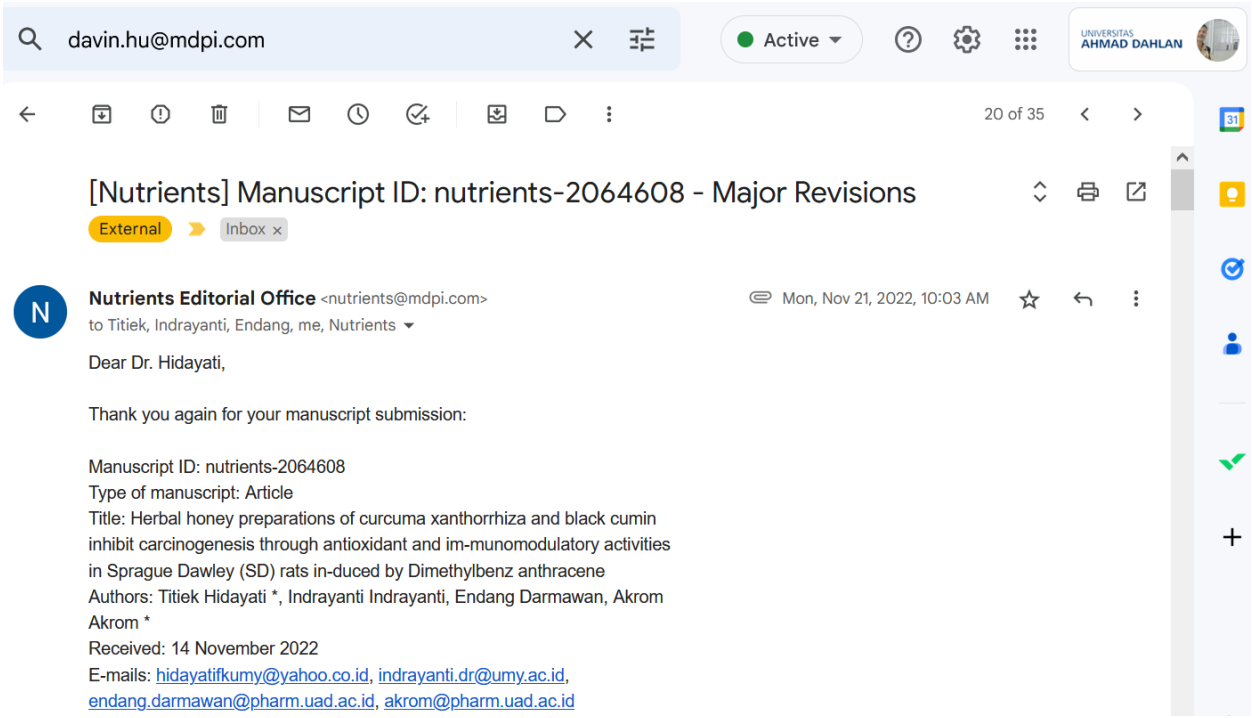
Please note that our invoice amount is fixed in Swiss Francs (CHF). If you need to pay in another currency, please note that the exchange rate of the invoice is fixed only when the editor confirms the invoice amount to the billing department.

Please also check and confirm that the below information for the invoice address is correct:

Name: Titiek - Hidayati
Address: Dr. Titiek - Hidayati
Universitas Muhammadiyah Yogyakarta
Public Health and Family medicine Department, Medicine and Health Science Faculty
Kapten Tendean no 59 kota madya Yogyakarta



Your manuscript has now been reviewed by experts in the field. Please find your manuscript with the referee reports at this link:

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Please revise the manuscript according to the referees' comments and upload the revised file within 10 days.

Please use the version of your manuscript found at the *attached* for your revisions.

(I) Please check that all references are relevant to the contents of the manuscript.
(II) Any revisions to the manuscript should be marked up using the *highlight* function if you are using MS Word/LaTeX, such that any changes can be easily viewed by the editors and reviewers.
(III) Please provide a cover letter to explain, *point by point*, the details of the revisions to the manuscript and your responses to the referees' comments.
(IV) If you found it impossible to address certain comments in the review reports, please include an explanation in your appeal.
(V) The revised version will be sent to the editors and reviewers.

If one of the referees has suggested that your manuscript should undergo extensive English revisions, please address this issue during revision. We propose that you use one of the editing services listed at https://www.mdpi.com/authors/english or have your manuscript checked by a native English-speaking colleague.

Do not hesitate to contact us if you have any questions regarding the revision of your manuscript. We look forward to hearing from you soon.

Kind regards,
Mr. Davin Hu
E-Mail: davin.hu@mdpi.com

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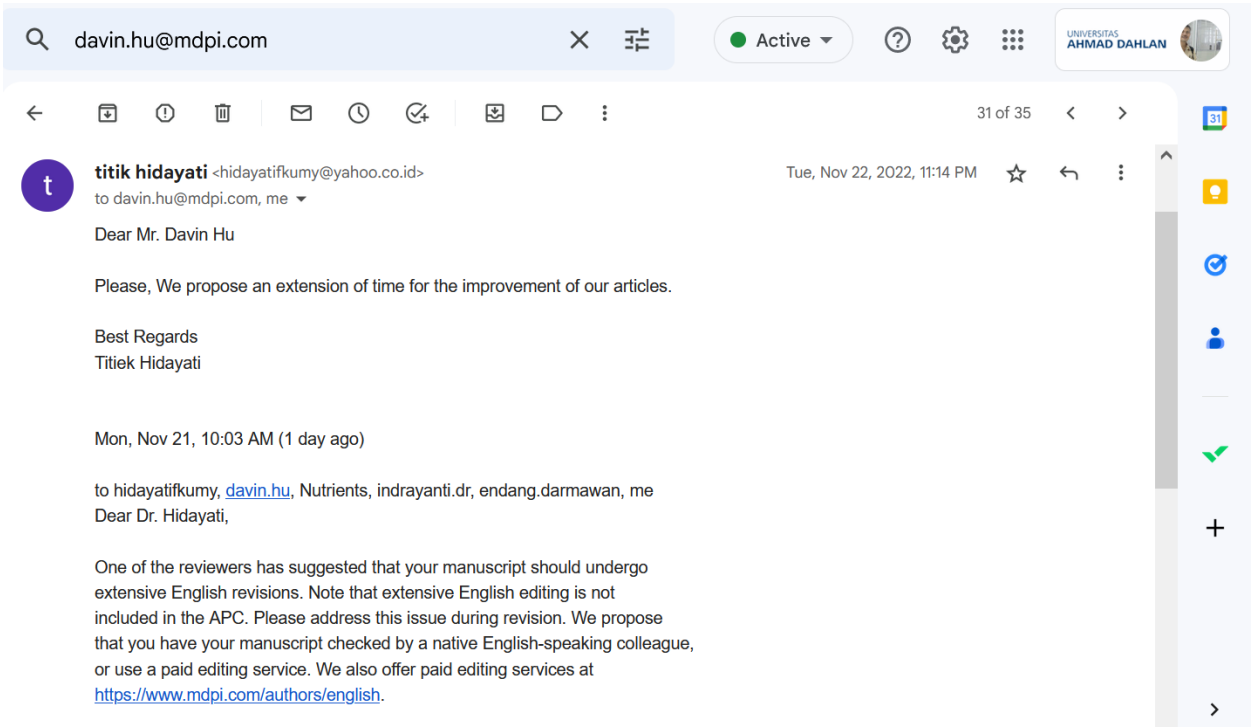


Mr Akrom yk <akrom@pharm.uad.ac.id>
to davin.hu

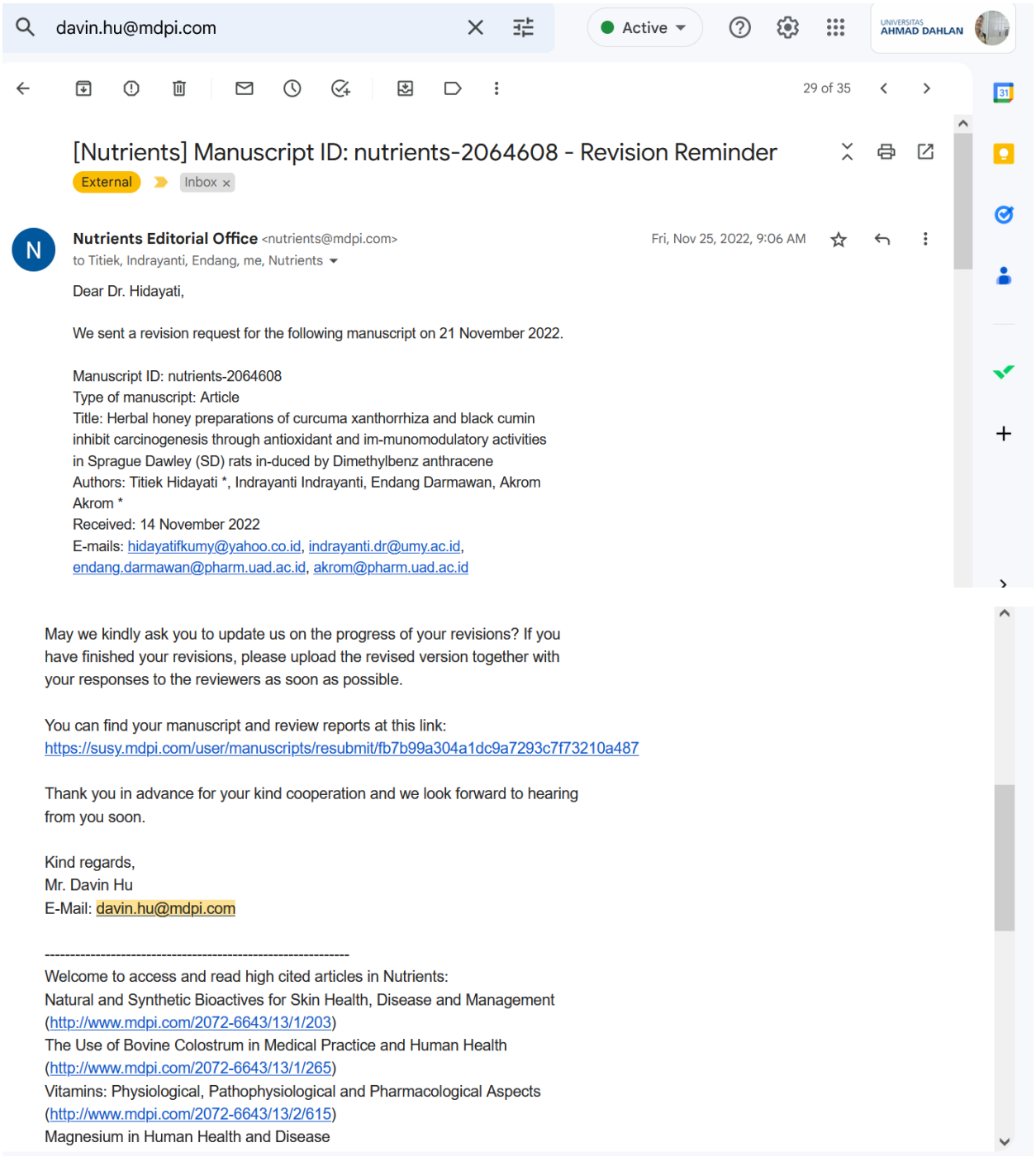
Tue, Nov 22, 2022, 11:17 PM

Dear Mr. Davin Hu

Please, We propose an extension of time for the improvement of our articles.

Best Regards

Permintaan revisi



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29 of 35

[Nutrients] Manuscript ID: nutrients-2064608 - Revision Reminder

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Nutrients Editorial Office <nutrients@mdpi.com>
to Titiek, Indrayanti, Endang, me, Nutrients

Fri, Nov 25, 2022, 9:06 AM

Dear Dr. Hidayati,

We sent a revision request for the following manuscript on 21 November 2022.

Manuscript ID: nutrients-2064608
Type of manuscript: Article
Title: Herbal honey preparations of curcuma xanthorrhiza and black cumin inhibit carcinogenesis through antioxidant and im-munomodulatory activities in Sprague Dawley (SD) rats in-duced by Dimethylbenz anthracene
Authors: Titiek Hidayati *, Indrayanti Indrayanti, Endang Darmawan, Akrom Akrom *
Received: 14 November 2022
E-mails: hidayatifkumy@yahoo.co.id, indrayanti.dr@umy.ac.id, endang.darmawan@pharm.uad.ac.id, akrom@pharm.uad.ac.id

May we kindly ask you to update us on the progress of your revisions? If you have finished your revisions, please upload the revised version together with your responses to the reviewers as soon as possible.

You can find your manuscript and review reports at this link:
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Thank you in advance for your kind cooperation and we look forward to hearing from you soon.

Kind regards,
Mr. Davin Hu
E-Mail: davin.hu@mdpi.com

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Vitamins: Physiological, Pathophysiological and Pharmacological Aspects (http://www.mdpi.com/2072-6643/13/2/615)
Magnesium in Human Health and Disease

Mr Akrom yk <akrom@pharm.uad.ac.id> Nov 25, 2022, 10:15 AM
to davin.hu

Dear Editor
Thank you, we are currently making revisions according to comments from reviewers. Thank you for your attention, since there are quite a lot of comments from reviewers and requires major revisions, would you like us to be given more time so that we can revise properly?

Best regard
Titiek H and Akrom

Reply Forward

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28 of 35

[Nutrients] Manuscript ID: nutrients-2064608 - Revision Reminder

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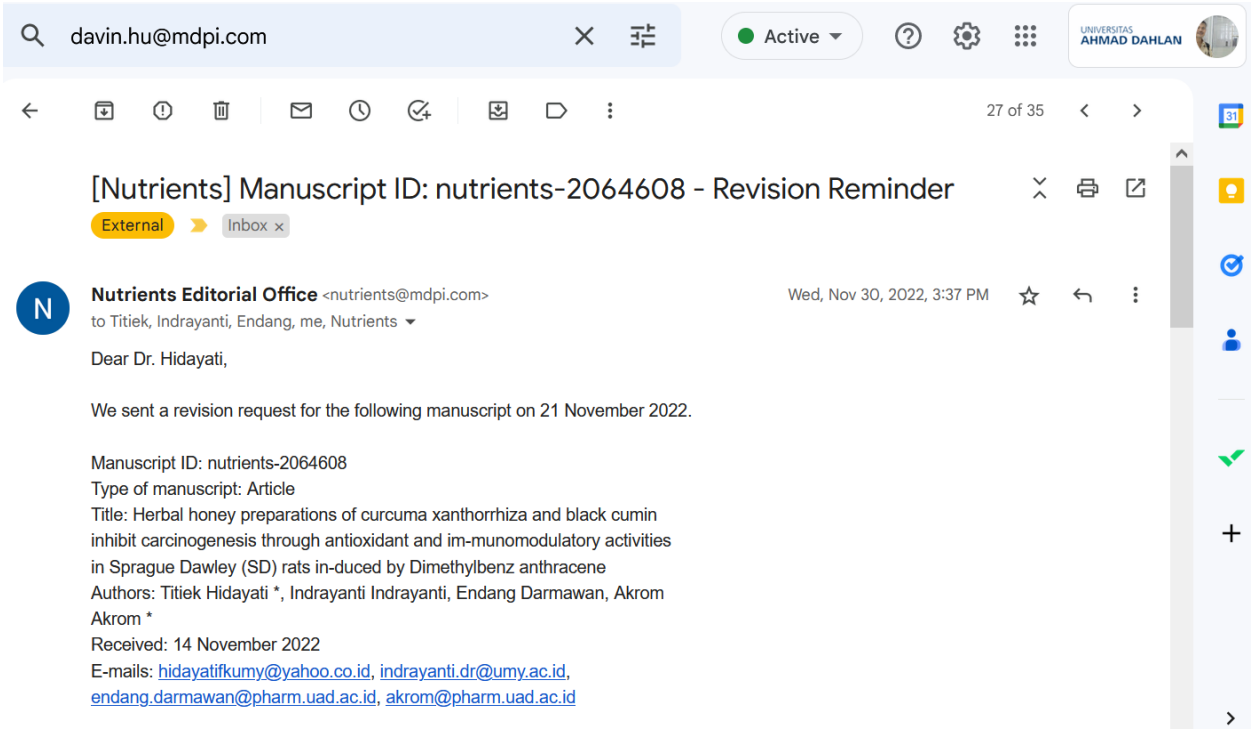
Nutrients Editorial Office <nutrients@mdpi.com> Mon, Nov 28, 2022, 11:15 AM
to Titiek, Indrayanti, Endang, me, Nutrients

Dear Dr. Hidayati,

We sent a revision request for the following manuscript on 21 November 2022.

Manuscript ID: nutrients-2064608
Type of manuscript: Article
Title: Herbal honey preparations of curcuma xanthorrhiza and black cumin inhibit carcinogenesis through antioxidant and im-munomodulatory activities in Sprague Dawley (SD) rats in-duced by Dimethylbenz anthracene
Authors: Titiek Hidayati *, Indrayanti Indrayanti, Endang Darmawan, Akrom Akrom *
Received: 14 November 2022
E-mails: hidayatifkumy@yahoo.co.id, indrayanti.dr@umy.ac.id, endang.darmawan@pharm.uad.ac.id, akrom@pharm.uad.ac.id



May we kindly ask you to update us on the progress of your revisions? If you have finished your revisions, please upload the revised version together with your responses to the reviewers as soon as possible.

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Thank you in advance for your kind cooperation and we look forward to hearing from you soon.

Kind regards,
Mr. Davin Hu
E-Mail: davin.hu@mdpi.com

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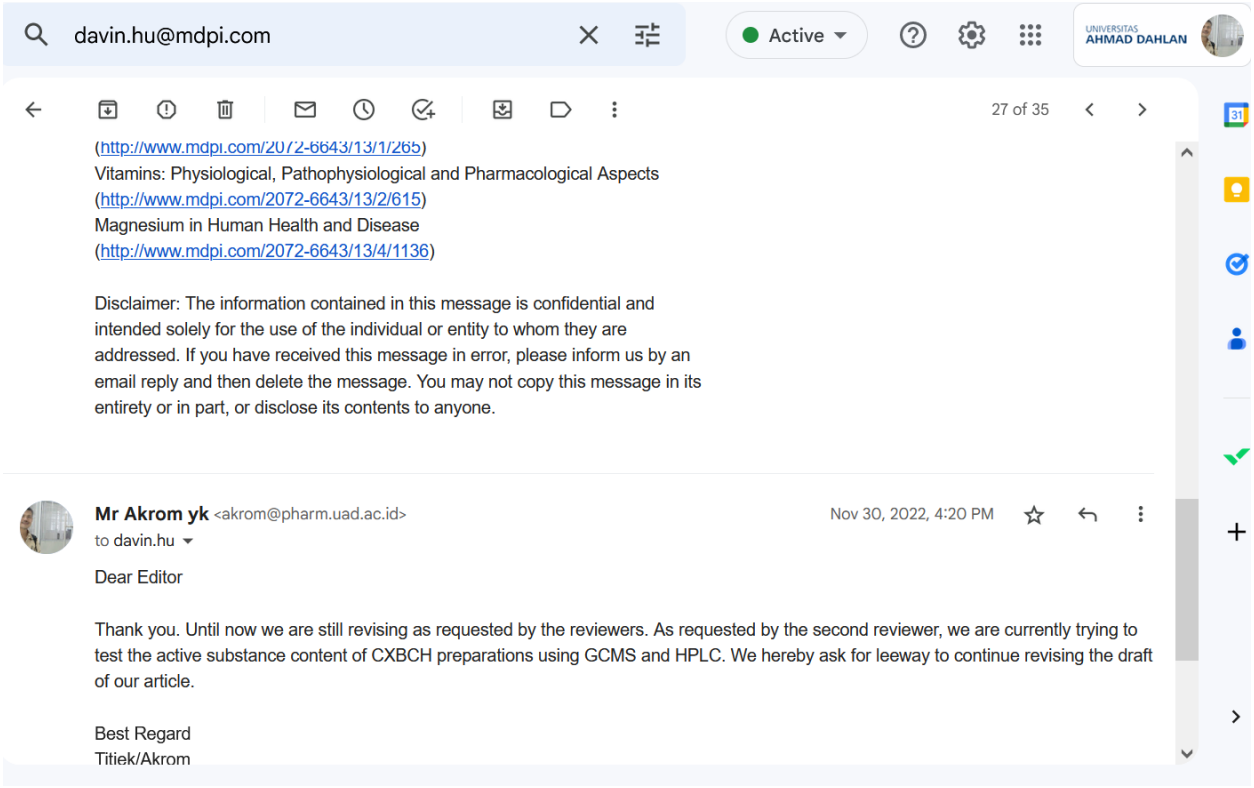
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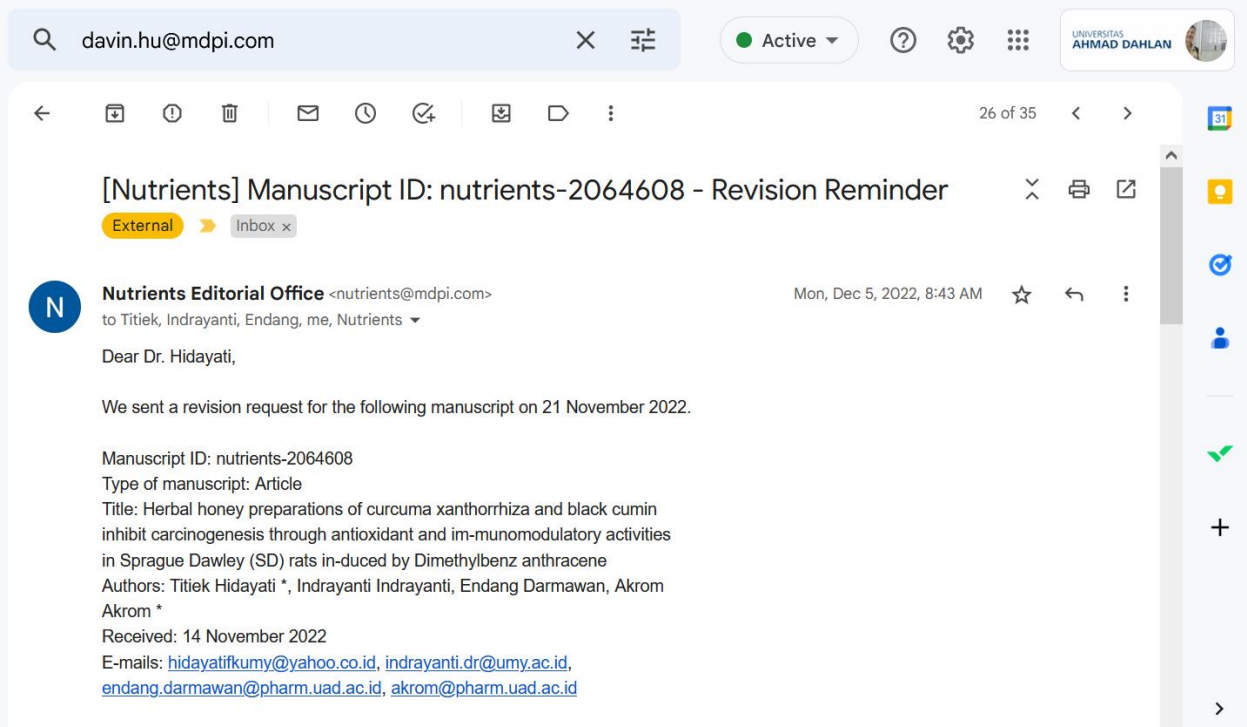
Mr Akrom yk <akrom@pharm.uad.ac.id>
to davin.hu
Nov 30, 2022, 4:20 PM
Dear Editor
Thank you. Until now we are still revising as requested by the reviewers. As requested by the second reviewer, we are currently trying to test the active substance content of CXBCH preparations using GCMS and HPLC. We hereby ask for leeway to continue revising the draft of our article.
Best Regard
Titiek/Akrom



May we kindly ask you to update us on the progress of your revisions? If you have finished your revisions, please upload the revised version together with your responses to the reviewers as soon as possible.

You can find your manuscript and review reports at this link:
https://susy.mdpi.com/user/manuscripts/resubmit/fb7b99a304a1dc9a7293c7f73210a487

Thank you in advance for your kind cooperation and we look forward to hearing from you soon.

Kind regards,
Mr. Davin Hu
E-Mail: davin.hu@mdpi.com

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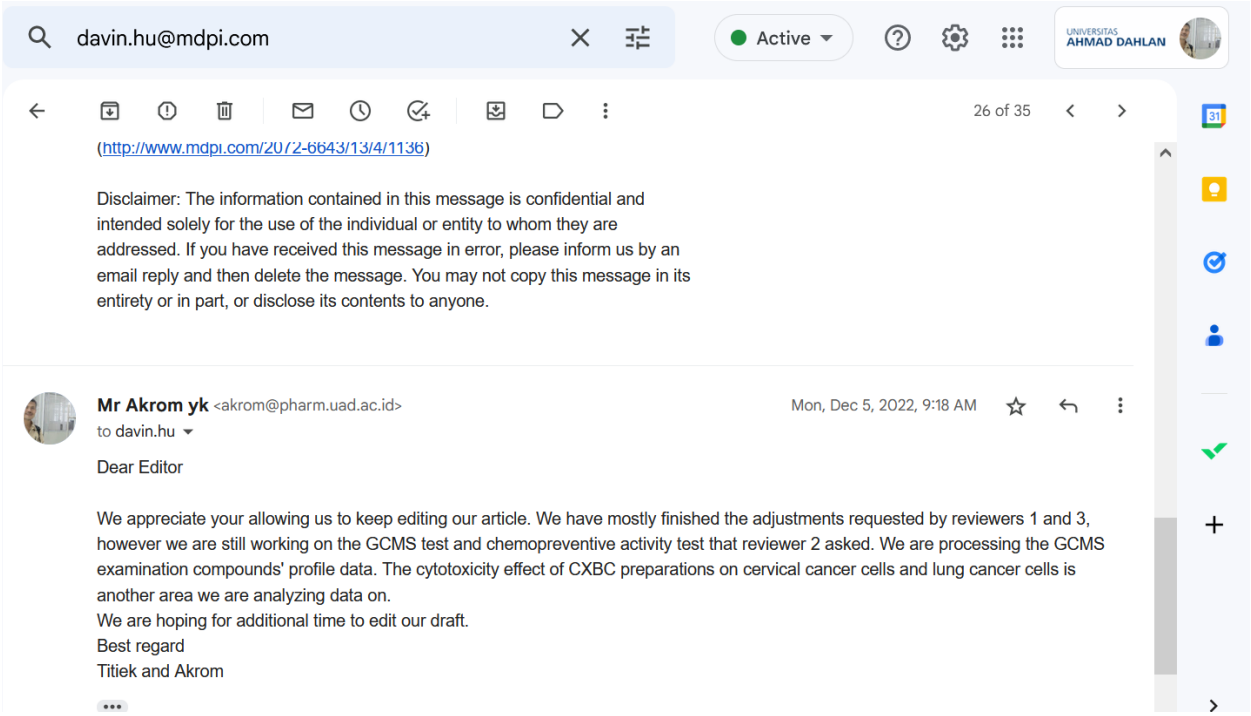
Vitamins: Physiological, Pathophysiological and Pharmacological Aspects (http://www.mdpi.com/2072-6643/13/2/615)
Magnesium in Human Health and Disease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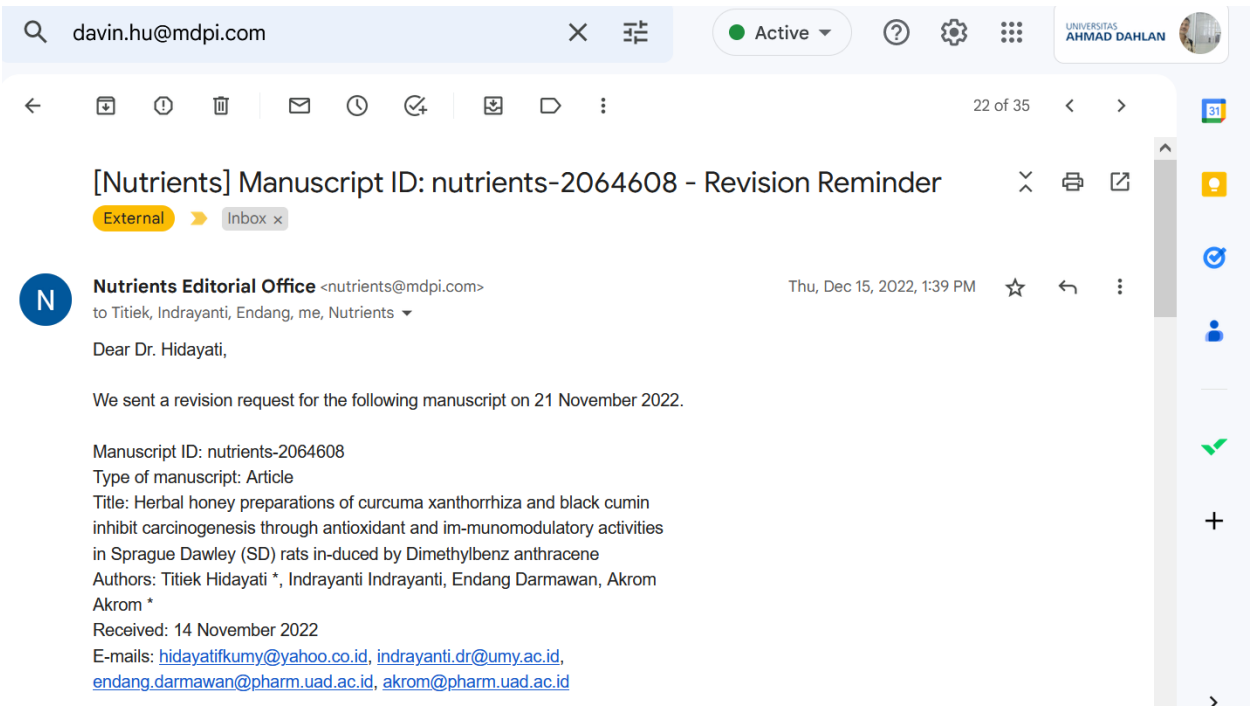
Mr Akrom yk <akrom@pharm.uad.ac.id>
to davin.hu
Mon, Dec 5, 2022, 9:18 AM
Dear Editor
We appreciate your allowing us to keep editing our article. We have mostly finished the adjustments requested by reviewers 1 and 3, however we are still working on the GCMS test and chemopreventive activity test that reviewer 2 asked. We are processing the GCMS examination compounds' profile data. The cytotoxicity effect of CXBC preparations on cervical cancer cells and lung cancer cells is another area we are analyzing data on.
We are hoping for additional time to edit our draft.
Best regard
Titiek and Akrom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[Nutrients] Manuscript ID: nutrients-2064608 - Revision Reminder
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Nutrients Editorial Office <nutrients@mdpi.com>
to Titiek, Indrayanti, Endang, me, Nutrients
Thu, Dec 15, 2022, 1:39 PM
Dear Dr. Hidayati,
We sent a revision request for the following manuscript on 21 November 2022.
Manuscript ID: nutrients-2064608
Type of manuscript: Article
Title: Herbal honey preparations of curcuma xanthorrhiza and black cumin inhibit carcinogenesis through antioxidant and im-munomodulatory activities in Sprague Dawley (SD) rats in-duced by Dimethylbenz anthracene
Authors: Titiek Hidayati *, Indrayanti Indrayanti, Endang Darmawan, Akrom Akrom *
Received: 14 November 2022
E-mails: hidayatifkumy@yahoo.co.id, indrayanti.dr@umy.ac.id, endang.darmawan@pharm.uad.ac.id, akrom@pharm.uad.ac.id

May we kindly ask you to update us on the progress of your revisions? If you have finished your revisions, please upload the revised version together with your responses to the reviewers as soon as possible.

You can find your manuscript and review reports at this link:
https://susy.mdpi.com/user/manuscripts/resubmit/fb7b99a304a1dc9a7293c7f73210a487

Thank you in advance for your kind cooperation and we look forward to hearing from you soon.

Kind regards,
Mr. Davin Hu
E-Mail: davin.hu@mdpi.com

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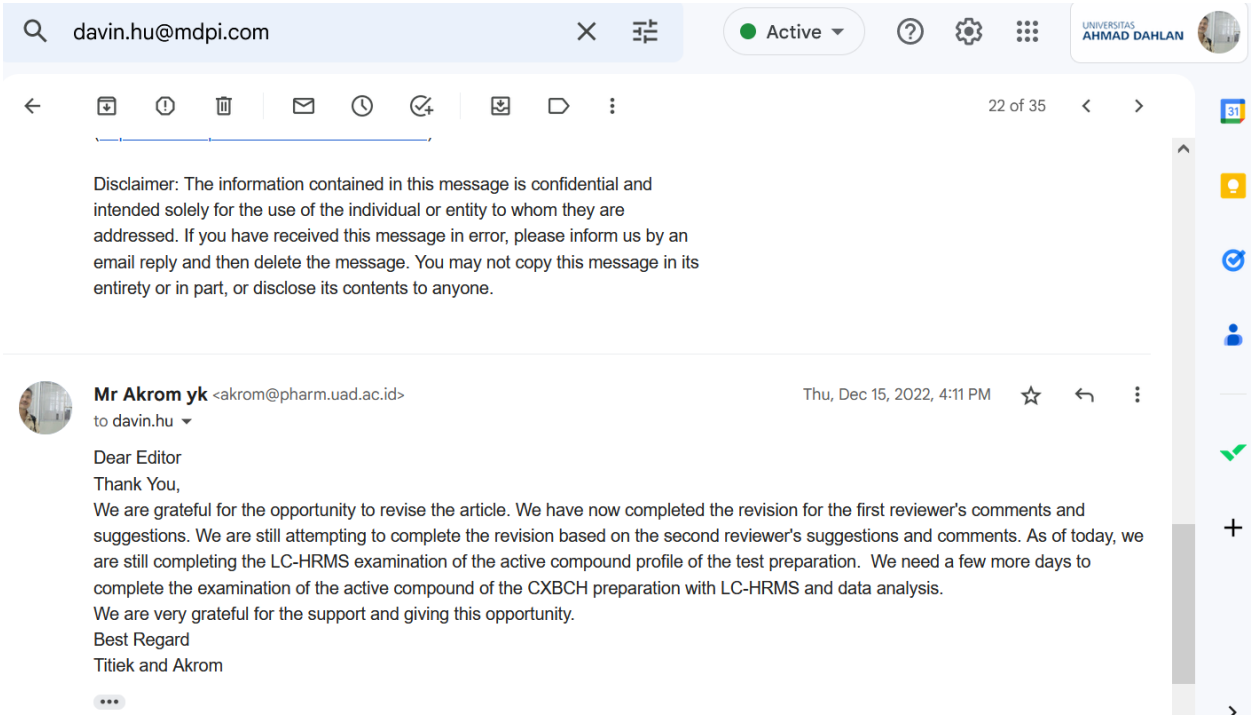
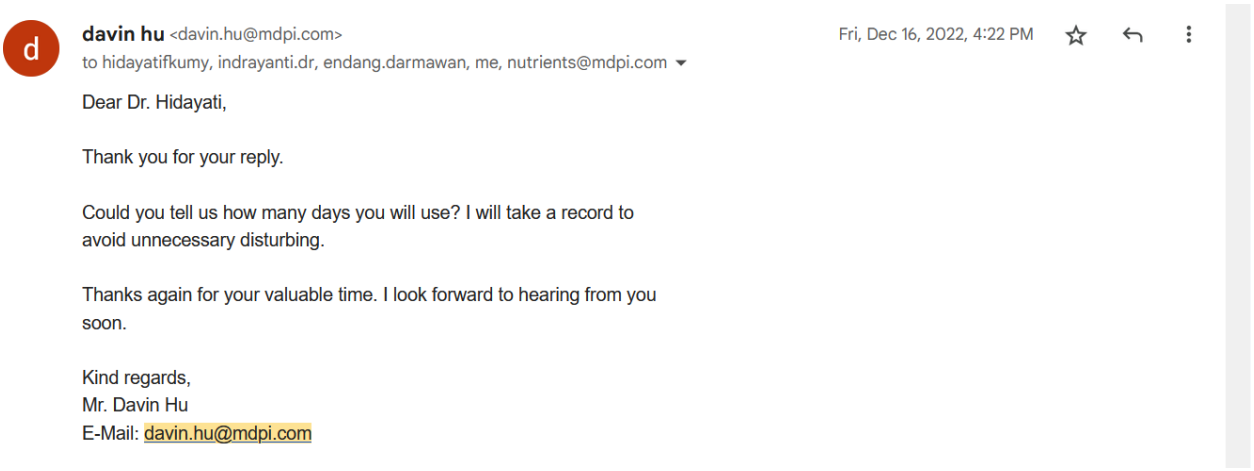
Vitamins: Physiological, Pathophysiological and Pharmacological Aspects
(http://www.mdpi.com/2072-6643/13/2/615)
Magnesium in Human Health and Disease



Peringatan untuk revisi dan jawaban permohonan tambahan waktu



davin hu <davin.hu@mdpi.com> Fri, Dec 16, 2022, 4:22 PM

to hidayatifkumy, indrayanti.dr, endang.darmawan, me, nutrients@mdpi.com

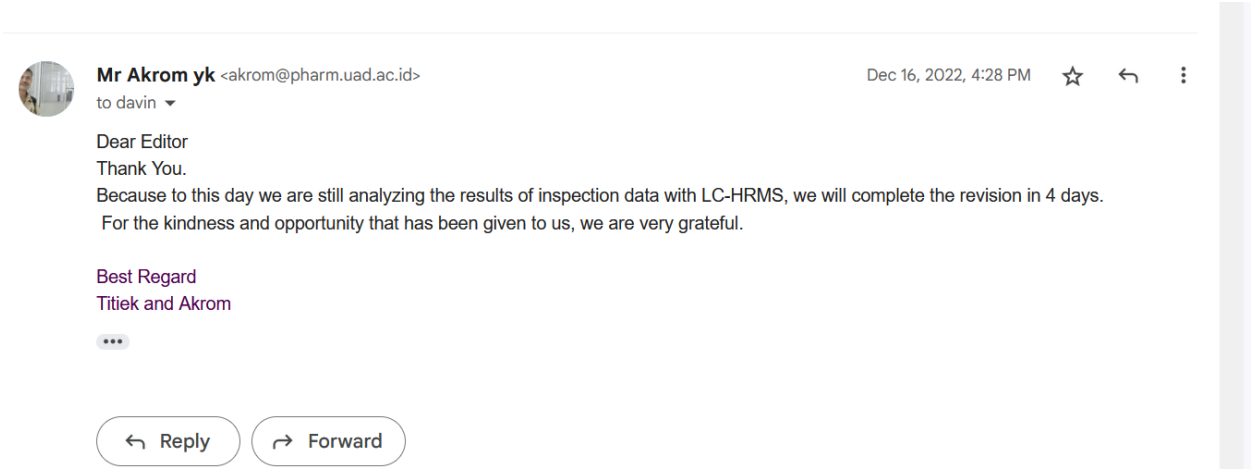
Dear Dr. Hidayati,

Thank you for your reply.

Could you tell us how many days you will use? I will take a record to avoid unnecessary disturbing.

Thanks again for your valuable time. I look forward to hearing from you soon.

Kind regards,
Mr. Davin Hu
E-Mail: davin.hu@mdpi.com



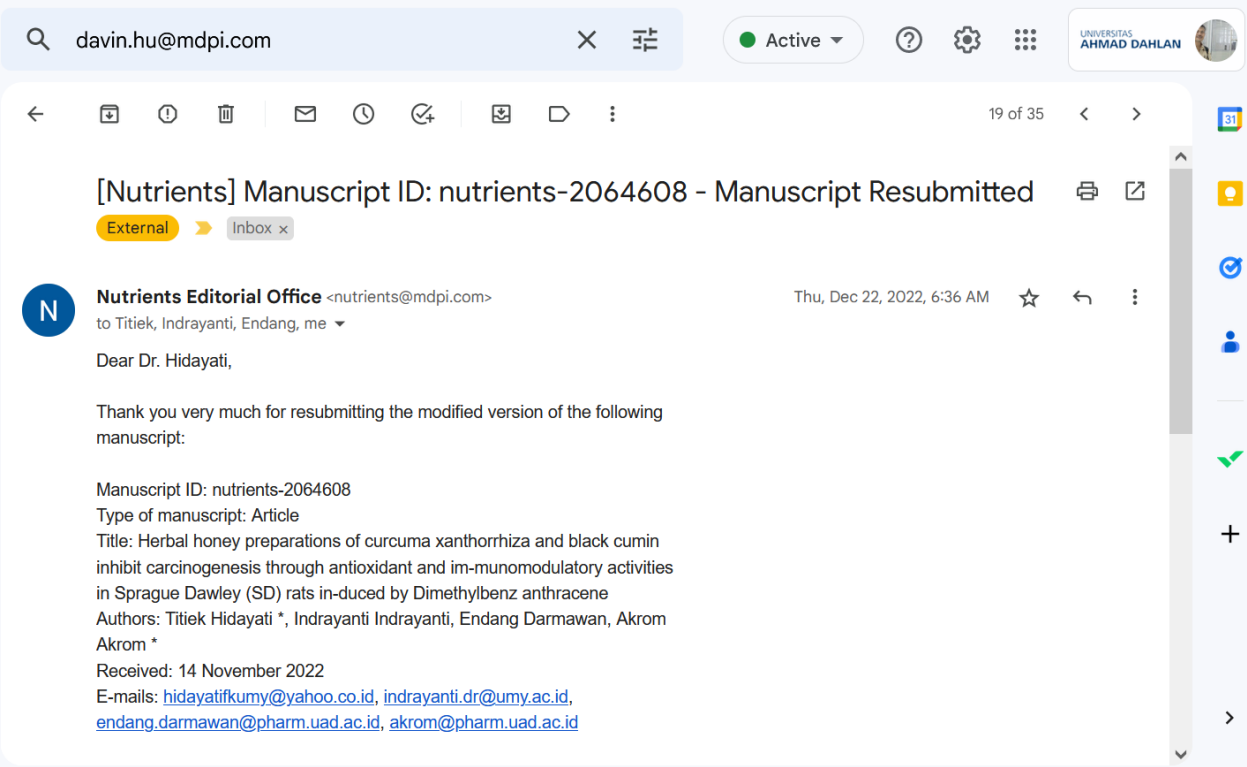
Mr Akrom yk <akrom@pharm.uad.ac.id> Dec 16, 2022, 4:28 PM

to davin

Dear Editor
Thank You.
Because to this day we are still analyzing the results of inspection data with LC-HRMS, we will complete the revision in 4 days.
For the kindness and opportunity that has been given to us, we are very grateful.

Best Regard
Titiek and Akrom

Reply Forward



Received: 14 November 2022
E-mails: hidayatifkumy@yahoo.co.id, indrayanti.dr@umy.ac.id, endang.darmawan@pharm.uad.ac.id, akrom@pharm.uad.ac.id

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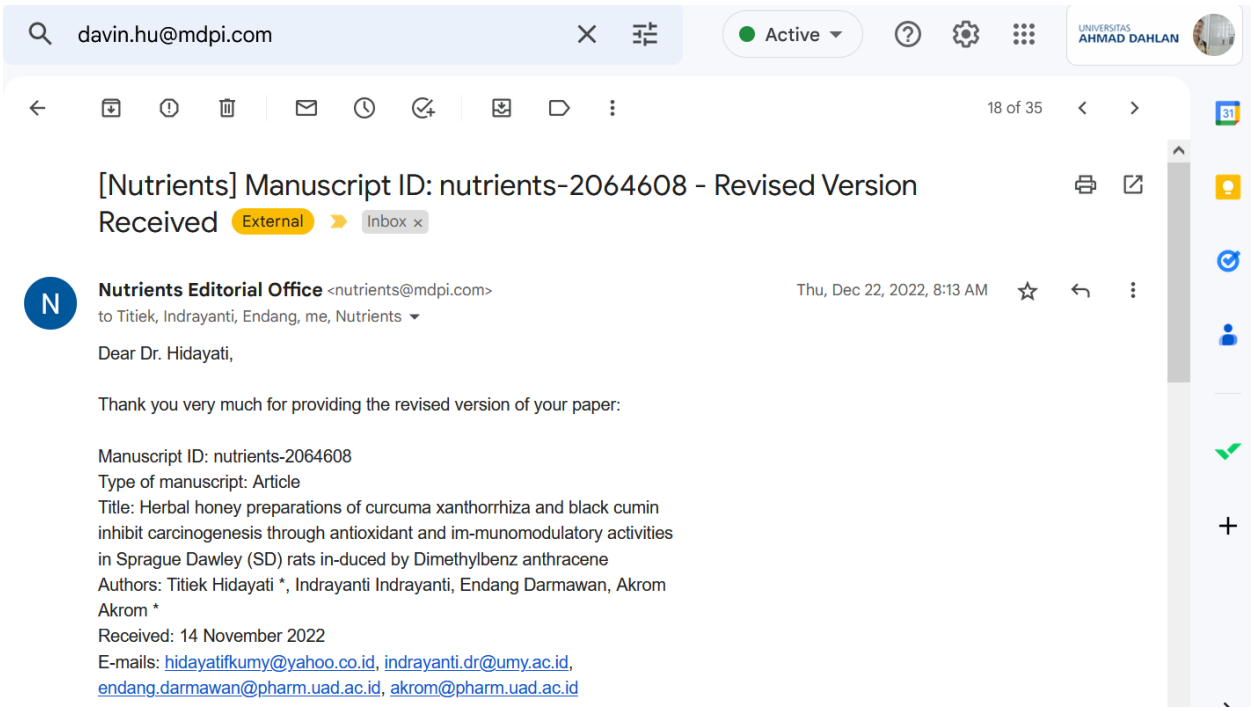
A member of the editorial office will be in touch with you soon regarding progress of the manuscript.

Kind regards,
Nutrients Editorial Office
Postfach, CH-4020 Basel, Switzerland
Office: St. Alban-Anlage 66, CH-4052 Basel
Tel. +41 61 683 77 34 (office)
E-mail: nutrients@mdpi.com
https://www.mdpi.com/journal/nutrients/



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[Nutrients] Manuscript ID: nutrients-2064608 - Revised Version Received

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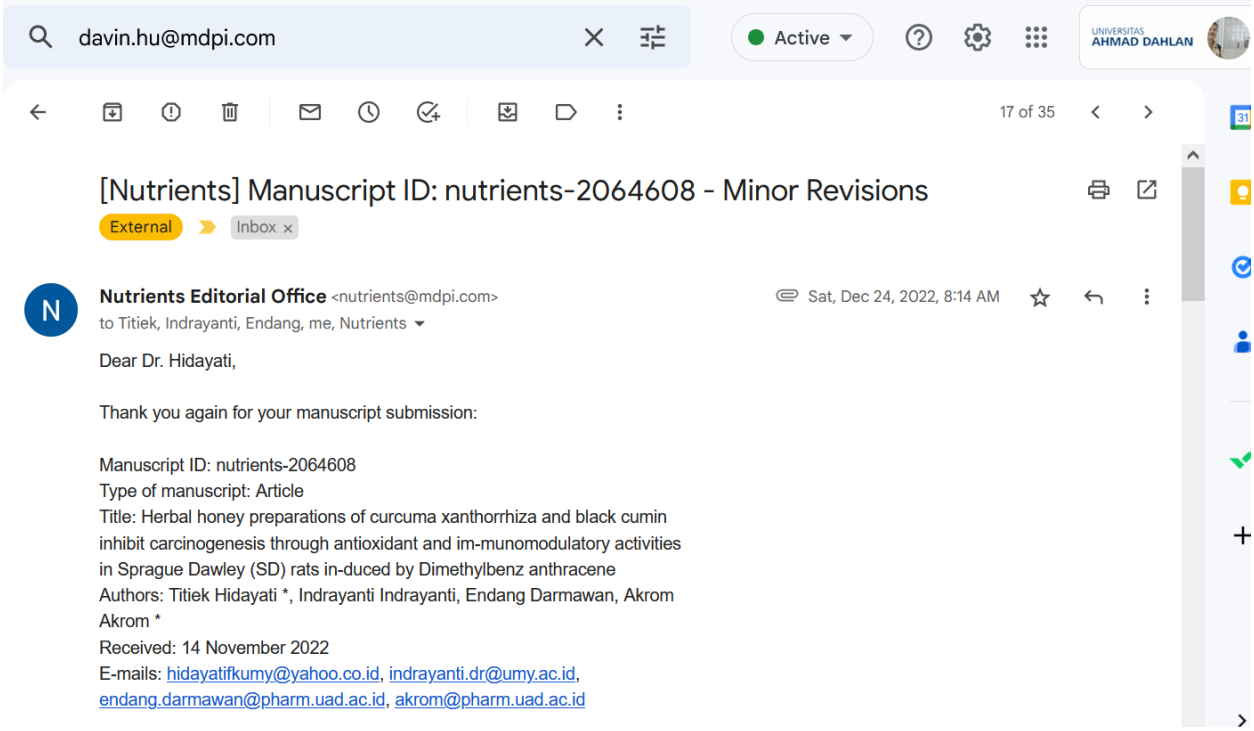
Nutrients Editorial Office <nutrients@mdpi.com>
to Titiek, Indrayanti, Endang, me, Nutrients

Thu, Dec 22, 2022, 8:13 AM

Dear Dr. Hidayati,

Thank you very much for providing the revised version of your paper:

Manuscript ID: nutrients-2064608
Type of manuscript: Article
Title: Herbal honey preparations of curcuma xanthorrhiza and black cumin inhibit carcinogenesis through antioxidant and im-munomodulatory activities in Sprague Dawley (SD) rats in-duced by Dimethylbenz anthracene
Authors: Titiek Hidayati *, Indrayanti Indrayanti, Endang Darmawan, Akrom Akrom *
Received: 14 November 2022
E-mails: hidayatifkumy@yahoo.co.id, indrayanti.dr@umy.ac.id, endang.darmawan@pharm.uad.ac.id, akrom@pharm.uad.ac.id



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[Nutrients] Manuscript ID: nutrients-2064608 - Minor Revisions

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Nutrients Editorial Office <nutrients@mdpi.com>
to Titiek, Indrayanti, Endang, me, Nutrients

Sat, Dec 24, 2022, 8:14 AM

Dear Dr. Hidayati,

Thank you again for your manuscript submission:

Manuscript ID: nutrients-2064608
Type of manuscript: Article
Title: Herbal honey preparations of curcuma xanthorrhiza and black cumin inhibit carcinogenesis through antioxidant and im-munomodulatory activities in Sprague Dawley (SD) rats in-duced by Dimethylbenz anthracene
Authors: Titiek Hidayati *, Indrayanti Indrayanti, Endang Darmawan, Akrom Akrom *
Received: 14 November 2022
E-mails: hidayatifkumy@yahoo.co.id, indrayanti.dr@umy.ac.id, endang.darmawan@pharm.uad.ac.id, akrom@pharm.uad.ac.id

Your manuscript has been reviewed by experts in the field. Please find your manuscript with the referee reports at this link:
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(I) Please revise your manuscript according to the referees' comments and upload the revised file within 2 days.
(II) Please use the version of your manuscript found at the *attached* for your revisions.
(III) Please check that all references are relevant to the contents of the manuscript.
(IV) Any revisions made to the manuscript should be marked up using the *highlight* function if you are using MS Word/LaTeX, such that changes can be easily viewed by the editors and reviewers.
(V) Please provide a short cover letter detailing your changes for the editors' and referees' approval.

If one of the referees has suggested that your manuscript should undergo extensive English revisions, please address this issue during revision. We propose that you use one of the editing services listed at https://www.mdpi.com/authors/english or have your manuscript checked by a native English-speaking colleague.

Please do not hesitate to contact us if you have any questions regarding the revision of your manuscript or if you need more time. We look forward to hearing from you soon.

Kind regards,
Mr. Davin Hu
E-Mail: davin.hu@mdpi.com

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https://www.mdpi.com/journal/nutrients/editors_choice

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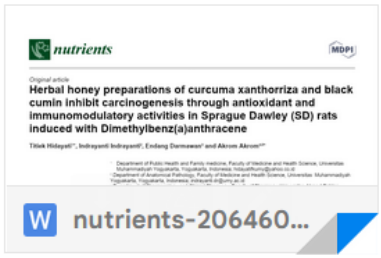

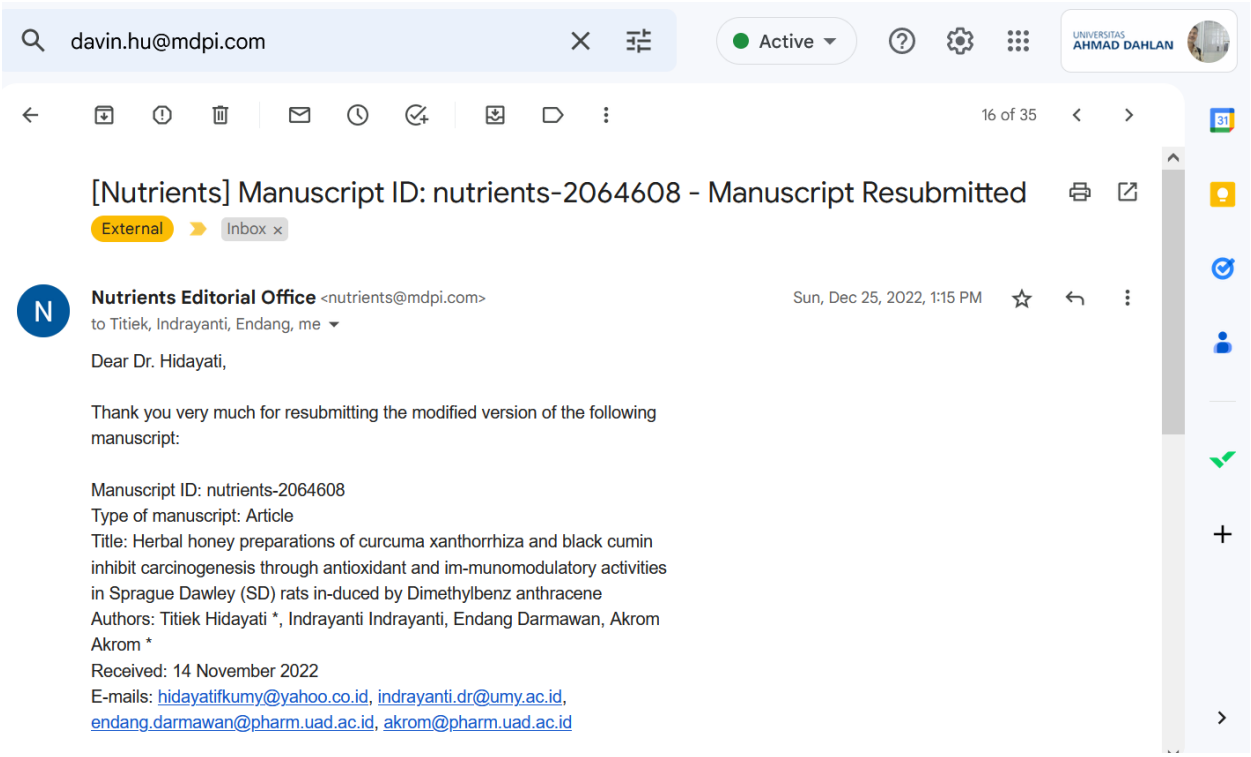

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A member of the editorial office will be in touch with you soon regarding progress of the manuscript.

Kind regards,
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Office: St. Alban-Anlage 66, CH-4052 Basel
Tel. +41 61 683 77 34 (office)
E-mail: nutrients@mdpi.com
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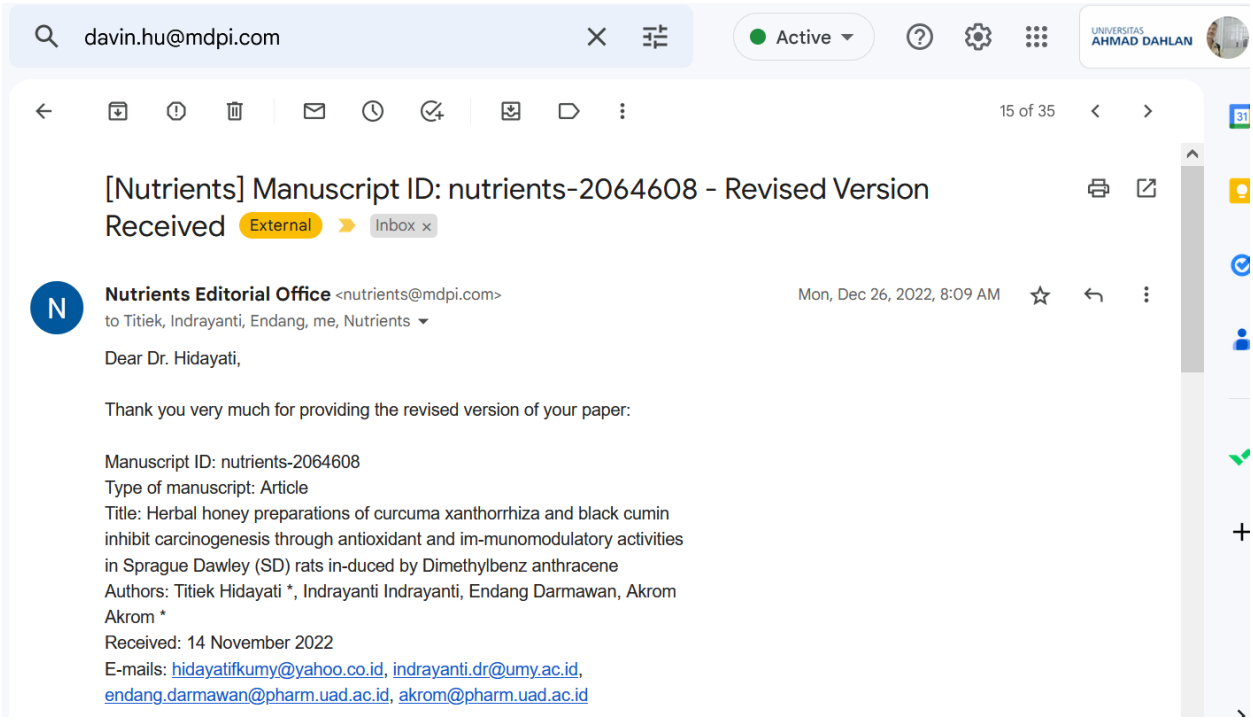

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E-Mail: davin.hu@mdpi.com

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3.One Giant Leap from Mouse to Man: The Microbiota–Gut–Brain Axis in Mood Disorders and Translational Challenges Moving towards Human Clinical Trials (http://www.mdpi.com/2072-6643/14/3/568)

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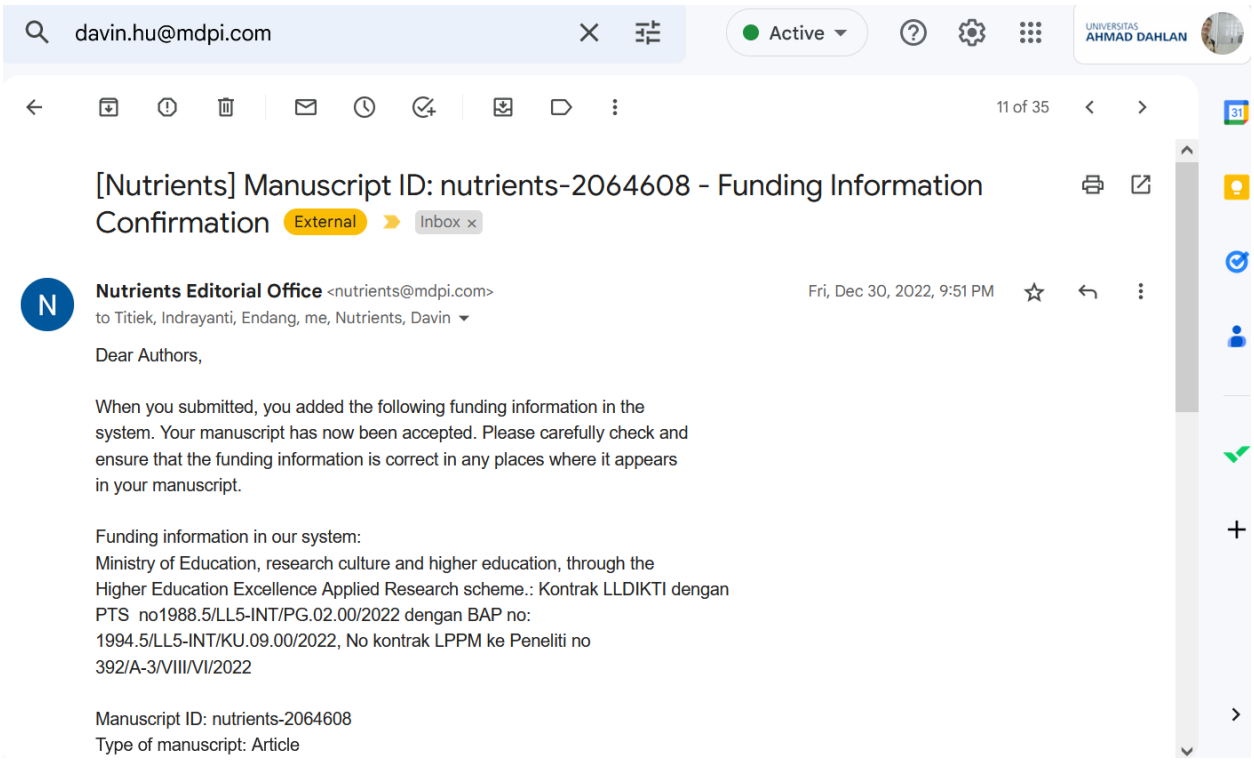

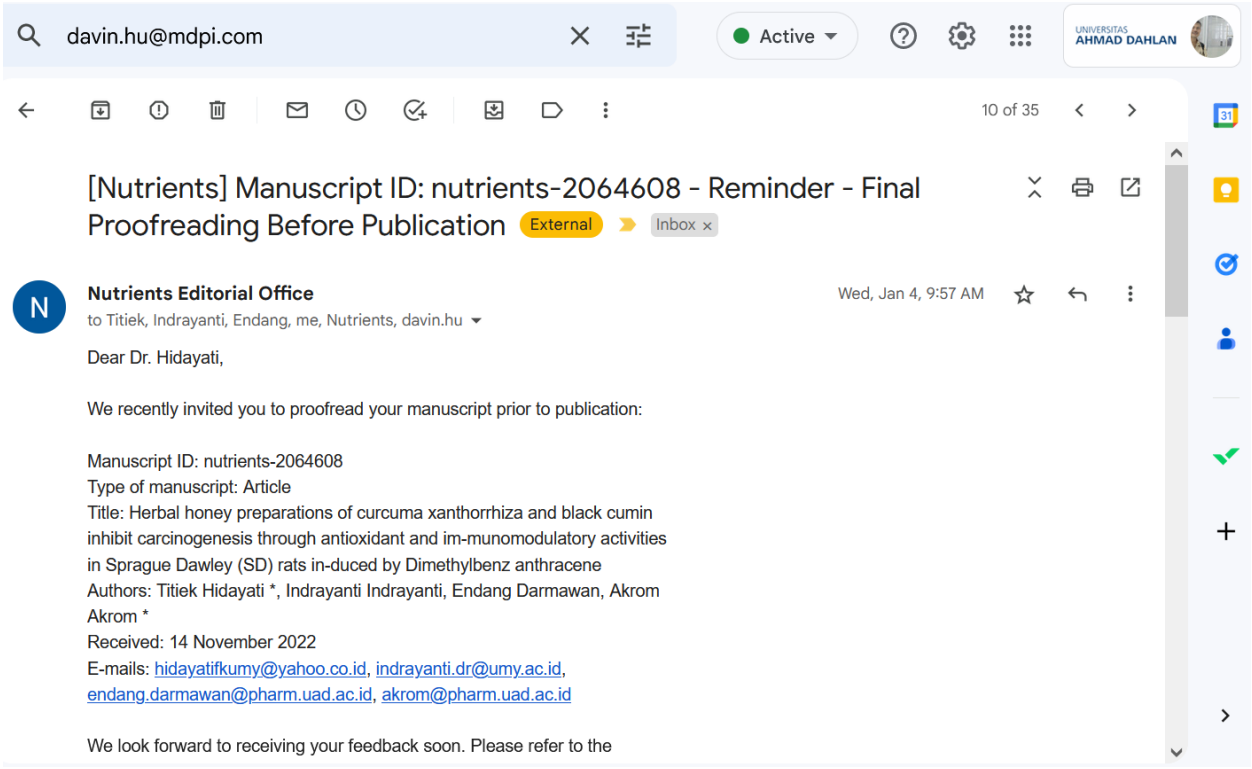

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UNIVERSITAS AHMAD DAHLAN

10 of 35

[Nutrients] Manuscript ID: nutrients-2064608 - Reminder - Final Proofreading Before Publication External Inbox ×

Nutrients Editorial Office
to Titiek, Indrayanti, Endang, me, Nutrients, davin.hu

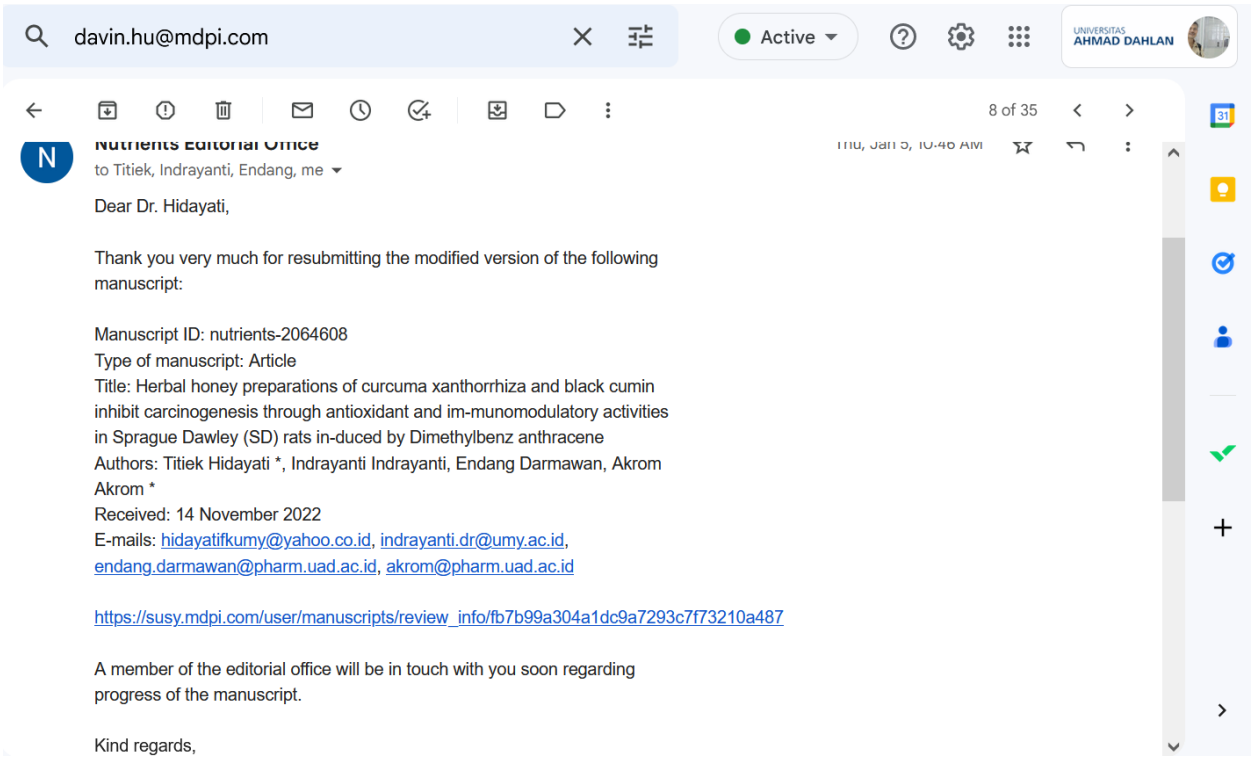
Wed, Jan 4, 9:57 AM

Dear Dr. Hidayati,

We recently invited you to proofread your manuscript prior to publication:

Manuscript ID: nutrients-2064608
Type of manuscript: Article
Title: Herbal honey preparations of curcuma xanthorrhiza and black cumin inhibit carcinogenesis through antioxidant and im-munomodulatory activities in Sprague Dawley (SD) rats in-duced by Dimethylbenz anthracene
Authors: Titiek Hidayati *, Indrayanti Indrayanti, Endang Darmawan, Akrom Akrom *
Received: 14 November 2022
E-mails: hidayatifkumy@yahoo.co.id, indrayanti.dr@umy.ac.id, endang.darmawan@pharm.uad.ac.id, akrom@pharm.uad.ac.id

We look forward to receiving your feedback soon. Please refer to the



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UNIVERSITAS AHMAD DAHLAN

8 of 35

Nutrients Editorial Office
to Titiek, Indrayanti, Endang, me

Thu, Jan 5, 10:46 AM

Dear Dr. Hidayati,

Thank you very much for resubmitting the modified version of the following manuscript:

Manuscript ID: nutrients-2064608
Type of manuscript: Article
Title: Herbal honey preparations of curcuma xanthorrhiza and black cumin inhibit carcinogenesis through antioxidant and im-munomodulatory activities in Sprague Dawley (SD) rats in-duced by Dimethylbenz anthracene
Authors: Titiek Hidayati *, Indrayanti Indrayanti, Endang Darmawan, Akrom Akrom *
Received: 14 November 2022
E-mails: hidayatifkumy@yahoo.co.id, indrayanti.dr@umy.ac.id, endang.darmawan@pharm.uad.ac.id, akrom@pharm.uad.ac.id

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A member of the editorial office will be in touch with you soon regarding progress of the manuscript.

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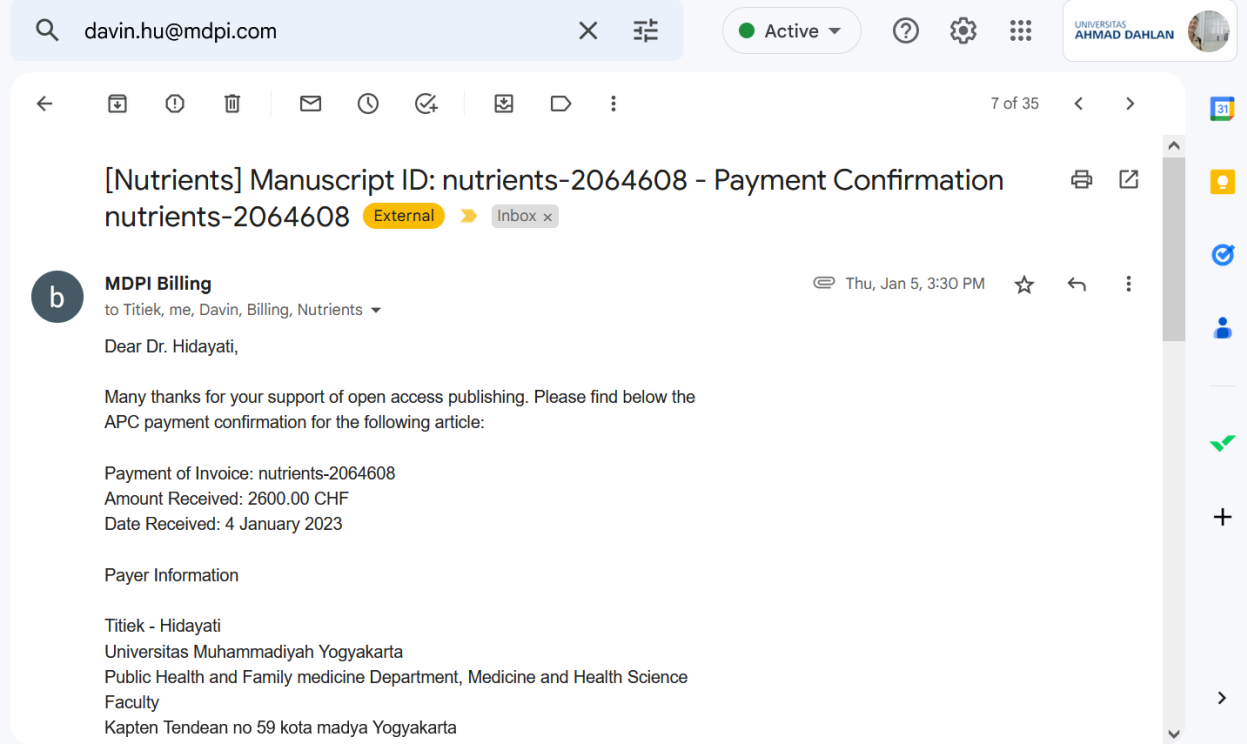

Titiek - Hidayati
Universitas Muhammadiyah Yogyakarta
Public Health and Family medicine Department, Medicine and Health Science Faculty
Kapten Tendean no 59 kota madya Yogyakarta
55252 Yogyakarta
Indonesia

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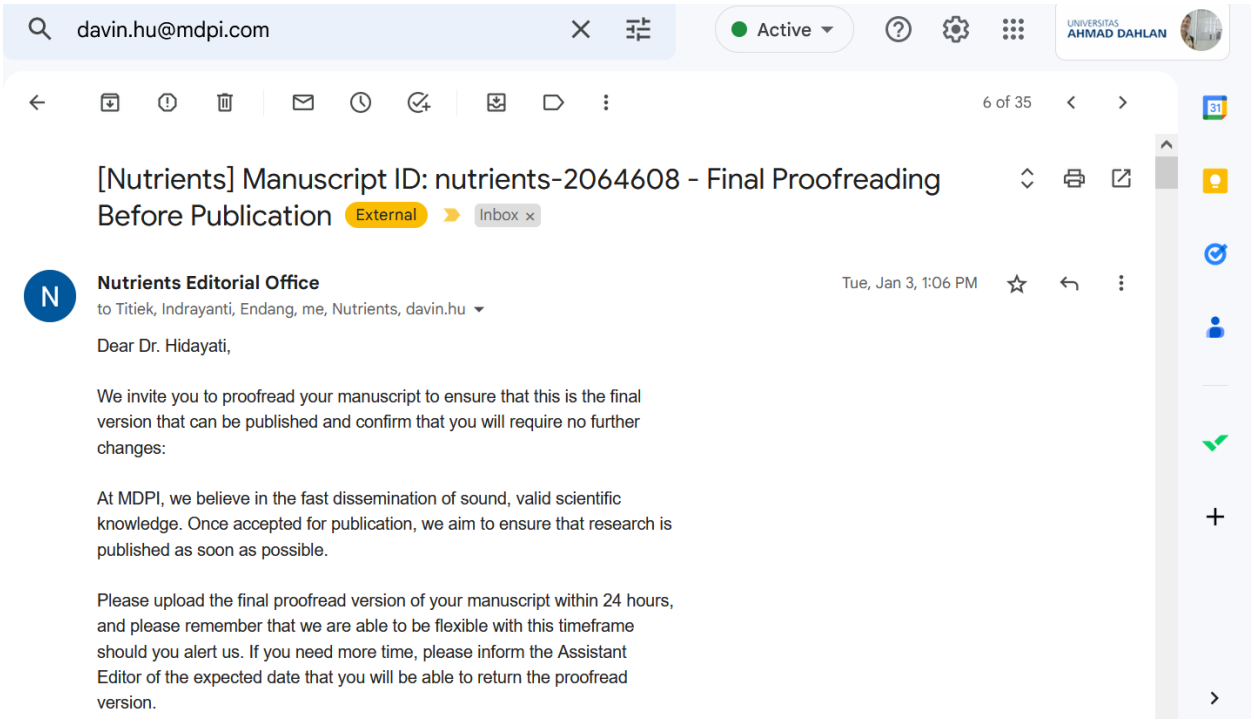

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Type of manuscript: Article
Title: Herbal honey preparations of curcuma xanthorrhiza and black cumin inhibit carcinogenesis through antioxidant and im-munomodulatory activities in Sprague Dawley (SD) rats in-duced by Dimethylbenz anthracene
Authors: Titiek Hidayati *, Indrayanti Indrayanti, Endang Darmawan, Akrom Akrom *
Received: 14 November 2022
E-mails: hidayatifkumy@yahoo.co.id, indrayanti.dr@umy.ac.id, endang.darmawan@pharm.uad.ac.id, akrom@pharm.uad.ac.id

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This manuscript includes supplementary materials, which you can find at the second link, above. Please note that citations and references in Supplementary files are permitted provided that they also appear in the reference list of the main text. Please ensure that you proofread your supplementary materials and upload them together with the manuscript.

We look forward to hearing from you soon.

Kind regards,
Mr. Davin Hu
Assistant Editor
E-Mail: davin.hu@mdpi.com

Welcome to access and read high cited articles in Nutrients:
1. The Potential Health Benefits of the Ketogenic Diet: A Narrative Review

---

davin hu Jan 5, 2023, 11:21 AM
to Titiek, Nutrients, Indrayanti, Endang, me

Dear Dr. Hidayati,

Thank you for your proofreading.

After checking, two problems still need to be solved.

(1)Please remove all the red wave lines in figure 1
(2)References [18] and [19] are repeated, please check. If they are same, and no new alternative references need to be added, please just confirm, then we will help you to remove the duplicate one. And rearrange the references.

You can find the paper in the attachment. After revising in the paper, you can kindly send it to us by this e-mail within four hours.

Thank you for your cooperation.

Kind regards,
Mr. Davin Hu
Assistant Editor

Thank you for your cooperation.

Kind regards,
Mr. Davin Hu
Assistant Editor
E-Mail: davin.hu@mdpi.com

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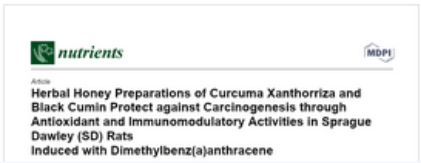
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Herbal Honey Preparations of Curcuma Xanthorriza and Black Cumin Protect against Carcinogenesis through Antioxidant and Immunomodulatory Activities in Sprague Dawley (SD) Rats Induced with Dimethylbenz(a)anthracene

Mr Akrom yk <akrom@pharm.uad.ac.id> Jan 5, 2023, 2:17 PM
to davin

Dear Editor

We have revised the draft according to the Editor's suggestion. Drafts attached.

Thank you

Best regard

Akrom

•••

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davin hu
to Titiek, Nutrients, Indrayanti, Endang, me

Jan 6, 2023, 8:27 AM

Dear Dr. Hidayati,

Thank you for your proofreading.

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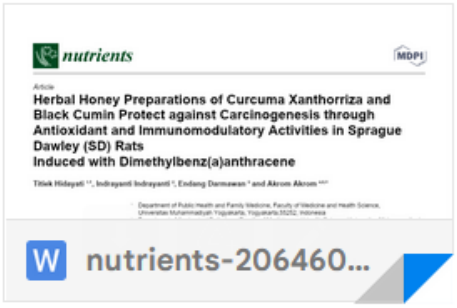

nutrients@mdpi.com
to Titiek, davin, Indrayanti, Endang, me

Jan 6, 2023, 3:30 PM

Dear Dr. Hidayati,

Thank you for your proofreading.

After checking, two problems still need to be solved as soon as possible.

(1)Please remove all the red wave lines in figure 1.
(2)References [18] and [19] are repeated, please check. If they are same, and no new alternative references need to be added, please just confirm, then we will help you to remove the duplicate one. And rearrange the references.

You can find the paper in the attachment. After revising in the paper, you can kindly send it to us by this e-mail.

Thank you for your cooperation.

Kind regards,
Amy Xie
Managing Editor

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Mr Akrom yk <akrom@pharm.uad.ac.id>
to davin, Titiek, Indrayanti, Endang

Jar

Thank you, I will do that.

Mr Akrom yk <akrom@pharm.uad.ac.id> Jan 6, 2023, 4:56 PM

to davin

Dear Editor

Herewith we attach the revised draft results.
Thank you.
Best Regard
Hidayati and Akrom

•••

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[Nutrients] Manuscript ID: nutrients-2064608; doi: 10.3390/nu15020371. Paper has been published.

External Inbox ×

nutrients@mdpi.com Wed, Jan 11, 7:36 PM

to hidayatifkumy, indrayanti.dr, endang.darmawan, me, billing, website, nutrients, marija.aleksic, davin.hu

Dear Authors,

We are pleased to inform you that your article "Herbal Honey Preparations of Curcuma Xanthorriza and Black Cumin Protect against Carcinogenesis through Antioxidant and Immunomodulatory Activities in Sprague Dawley (SD) Rats Induced with Dimethylbenz(a)anthracene" has been published in Nutrients and is available online:

Website: https://www.mdpi.com/2072-6643/15/2/371
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davin hu Thu, Jan 12, 9:06 AM

to hidayatifkumy, indrayanti.dr, endang.darmawan, me, nutrients

Dear Authors,

Congratulations that your paper "Herbal Honey Preparations of Curcuma Xanthorriza and Black Cumin Protect against Carcinogenesis through Antioxidant and Immunomodulatory Activities in Sprague Dawley (SD) Rats Induced with Dimethylbenz(a)anthracene" has been published in Nutrients. Please carefully check your paper (Website: https://www.mdpi.com/2072-6643/15/2/371). If there are incorrect contents, please inform us within 24 hours. Please kindly understand in this stage we do not accept any changes in linguistic issues.

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[Nutrients] Manuscript ID: nutrients-2064608 - Accepted for Publication

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Nutrients Editorial Office <nutrients@mdpi.com> Fri, Dec 30, 2022, 9:50 PM

to Titiek, Indrayanti, Endang, me, Nutrients, Davin

Dear Dr. Hidayati,

Congratulations on the acceptance of your manuscript, and thank you for submitting your work to Nutrients:

Manuscript ID: nutrients-2064608
Type of manuscript: Article
Title: Herbal honey preparations of curcuma xanthorrhiza and black cumin inhibit carcinogenesis through antioxidant and im-munomodulatory activities in Sprague Dawley (SD) rats in-duced by Dimethylbenz anthracene
Authors: Titiek Hidayati *, Indrayanti Indrayanti, Endang Darmawan, Akrom Akrom *
Received: 14 November 2022
E-mails: hidayatifkumy@yahoo.co.id, indrayanti.dr@umy.ac.id, endang.darmawan@pharm.uad.ac.id, akrom@pharm.uad.ac.id

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Yogyakarta 55252
Indonesia

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Dear Reviewer

We appreciate your insightful and motivating comments and recommendations for strengthening our manuscript.

Our draft paper has been amended to reflect your views and ideas. A draft of our essay has been sent to a qualified linguist. The certificate of proofreading is included.

The attached point-by-point revision list.

Thank you

Best regard

Titiek and Akrom

comment and Feedback from Reviewer	Answer and Correction
many important publications that should be cited were missing. For example, - DMBA is converted to DMBA-3,4-diol-1,2-epoxide by cytochrome P450 enzymes 1A1 or 1B1 (CYP1A1 or CYP1B1) and microsomal hydrolase enzymes (DMBA-DE). (Line 44) DMBA-DE is a genotoxic and immunosuppressive active metabolite of DMBA. (Line 46)	Thank you for the advice and remarks. We are aware that the draft currently lacks numerous references that we have found. A few references have been included in accordance with the citation. When we changed it, the number of references increased from 60 to 96.
2) the average amounts of DMBA existed in the cigarette smoke and vehicle engine fumes should be added in the introduction section.	Information about the PAH content of cigarettes and the concentrations of benz anthracene in exhaust gases from moving vehicles has been included.(line 43 to line 52)
3) what are the difference between “moderate amounts (Line 48)” and “excessive amounts (Line 50)”?	Thanks for the comments. “Moderate amounts” is physiologically or normal value. We've corrected "moderate amounts" to "normal value or physiologically".(line 60-61)
4) In many places, the manuscript is written in a weak English language. This manuscript is not reached for evaluation.	Thank you for the comments. We have submitted articles for proof reading to professional editors.
5) what is “WT” meaning? (Line 52)	Thank “WT mice” =”wild tipe (WT) mice” (line 64)
6) what is “active radicals” meaning? (Line 84) Are there “inactive radicals”?	Thanks, thanks for the comments. We mean “Active radicals” is “free radicals”. We've fixed it. (line 96)
7) detail information about “the traditional herbal medicine industry certified by the Food and Drug Supervisory Agency (Line 112)” should be added.	Thanks "The traditional herbal medicine industry certified by the drug supervisory agency (FDSA)". FDSA has granted a certificate for good manufacturing practice (GMP) to CV Al Afiat. CV Al Afiat is a form of Small Business Traditional Medicine (Jamu) in Indonesia.(line 203-204)
8) who is the “experts”? (Line 114)	Prof. Dr. Subagus Wahyuono, Apt. An expert in pharmaceutical biology from the Faculty of Pharmacy, Gadjah Mada University. (line 126)

9) where did the authors obtain T47D cells used in this study? (Line 122)	We got cancer cell line (T47D, and Hela cells thanks to Prof. Dr. Edy Meianto, Apt. from The "Cancer and Chemoprevention research Center" Gadjah Mada University. (line 137-138)
10) why did the authors used female animals? (Line 124)	Thanks for the comments. In accordance with earlier studies, we used female test animals to undertake a chemopreventive test of the DMBA chemical carcinogen model. Female Sprague Dawley rats that are four to six weeks old have shown that DMBA causes cancer. (line 147-148)
11) what was the "EAPU"? (Line 126)	Thanks for the correction. We have corrected the draft, "We used 80 female Sprague Dawley rats aged 24 – 30 days with an average weight of 80-120 g obtained from the Preclinical and Experimental Animal Development Unit (PEADU), Gadjah Mada University." PEADU is a unit providing experimental animals from Gadjah Mada University." (line 139-142)
12) detail information about animal housing room, including temperature, humidity and light/dark cycle should be added. (Line 128).	80 – 120 g female Sprague Dawley rats were purchased from the Preclinical Experiment and Animal Development Unit (PEADU), Gadjah Mada University, Yogyakarta, Indonesia, when they were between four and six weeks old. The animals were kept in standardized climatic settings (22–28°C, 60–70% relative humidity, and a 12-hour cycle of darkness and light). They were kept in properly ventilated cages and given access to unlimited amounts of water as well as pelleted food (brailer-II, Japfa Comfeed Ltd). All animal experiments were conducted in accordance with the guidelines established by Universitas Ahmad Dahlan's ethical research committee. (line 149-153)
13) detail composition of diet used in this study should be added (Line 128).	Thank you Japfa Comfeed Ltd.'s standard feed was ordered. Rats are often fed on brailler-II pellets (BR-II), which are made from a

	combination of corn, soybean meal, wheat germ, coconut meal, fish meal, meat meal, rice flour, tapioca, and premixes of coconut oil and fish oil. (line 154-157)
14) detail information about company, such as Ohaus and Cosmos, should be added. (Line 131)	Thanks for the comments and feed back. We have added the company of each research tool and material (line 159-199)
15) the authors stated that "The quantities of thymoquinone, curcumin, polyphenols, and flavonoids in CX extract, BC extract, and CXBC preparations were determined using thymoquinone (Sigma), curcumin (Sigma), gallic acid (Sigma), and rutin (Sigma) standards. (Line 135)." Detail information about how to determine these compounds must be added.	Thank you for the comments and feedback. We write an explanation regarding the assay procedure in the inspection sub-procedure. (line 210-287)
16) what is the "predetermined composition" meaning? (Line 173)	Thank you The meaning "the predetermined composition"is composition formulation of CXBCH preparation (line 207)
17) what is the "certain speed" meaning? (Line 174)	'constant speed" (line 208)
18) where did the authors obtain the "Folin-Ciocalteau reagent"? (Line 179)	Folin-Ciocalteu reagent (Merck, Germany) (line 220)
19) the authors stated that "1 mL of 50,000 ppm sample solution was pipetted and placed in a 10 mL volumetric flask (Line 191)." Here, 50,000 ppm is 5g/100mL (5%). Is this the correct information? The sample was dissolved as concentration of 5g/100mL?	Thank you We've revised the draft. We prepared a sample stock solution of the test preparation with a concentration of 5%. 500 mg of sample was put in a 10 mL volumetric flask then added with aqua until it reached the limit of 10 mL and then homogenized. (line 231-239)

20) the authors stated that "then quantified using a UV-Visible spectrophotometer at a wavelength of 200 – 400 nm. (Line 203)" How to analyze the amounts using such range of wavelength?	"then quantified using a UV-Visible spectrophotometer at a wavelength of 200 – 400 nm to find out the specific wave length number of thymoquinone" (line 247-249)
21) the authors stated "Calibration curves were made using a series of reference standard solutions with five different concentrations (0.5, 1, 2, 5, and 10 g/mL). (Line 218)" Is this the correct information? In my opinion, it is impossible to prepare the standard solution with such a higher concertation (10 g/mL).	It should be 0.5, 1.0, 2.0, 5.0, and 10.0 microgram/mL (line 255, 264)
22) the authors stated that "T47D cells were cultured in 2% FBM supplemented with 1% penicillin (100 units/mL) and streptomycin (100 g/mL) at 37 °C, 5% CO2 in an incubator. (Line 247)" It might be impossible to use such concentration (100g/mL) of streptomycin.	"T47D cells were cultured in 2% FBM supplemented with 1% penicillin (100 units/mL) and streptomycin (100 ug/mL) at 37 °C, 5% CO2 in an incubator.(line 305-309)
23) the authors stated that "A 200 L of DMSO reagent was added to dissolve the formazan product in each well (Line 253)." I cannot understand, why the authors added such huge amount of DMSO (200L) in the well.	"A 200 uL of DMSO reagent was added to dissolve the formazan product in each well (line 313)
24) the authors stated that "Group II was given CXBC1 (equivalent to 1x5 ml/70kg BW). (Line 260)," indicating that the treatment amount was 0.07 mL/kg body weight. In this study, the body weight of rats were a few 100 g. In my opinion, it is quite difficult to inject such low amount (about 10 uL/animal).	Many thanks We changed the provided dose from a 70kg human dose to a dose for rats (200 grams): CXBCH1=0.018 x 5 ml= 0.09 ml CXBC CXBCH2=0.018 x 10 ml= 0.18 ml CXBC CXBCH3=0.018 x 15 ml =0.27 ml CXBC

	To create a CXBC preparation solution that comprises 0.09 ml of CXBC/1 ml of volum solution, we diluted the CXBC preparation using aqua. 100 ml of aqua were added after 1 ml of CXBC, and the mixture was then agitated until it was homogenous. Because each set of test animals weighed 100 grams, a solution comprising 0.09 mL/1 mL of CXBC was administered along with volumes of 1/2 mL, 1 mL, and 1.5 mL of CXBCH1, CXBCH2, and CXBCH3. (Line 321-327)
25) detail information about "Integrated Testing and Examination Institute unit I (Line 283)" should be added.	Experimental Animal Service Unit of Integrated Testing and Examination Institute of Gadjah Mada University, Indonesia (line 346-347)
26) detail information about "Sysmeix kx 12 hematology analyzer (Line 285)" should be added.	hematology analyzer (Sysmex kx 12, Sysmex Ltd. Indonesia) (line 348)
27) what kind of anesthesia was used sacrifice?	the rats were sacrificed using chloroform vapor. (line 374)
29) "rpm" should be "g (gravity)" for example, Line 292.	1,789 g (line 355)
30) the error bar was missing on Figure 2.	We've added an error bar (line 553-570)
31) detail data about body weight and food intake during the experiments must be added	Animal body weight 100-300 g. The animal food was BR2 (Japfa Comfeed Ltd.) (line 154-157)

nutrients

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Original article

Herbal honey preparations of curcuma xanthorriza and black cumin inhibit carcinogenesis through antioxidant and immunomodulatory activities in Sprague Dawley (SD) rats induced with Dimethylbenz(a)anthracene

Titiek Hidayati[1]*, Indrayanti Indrayanti[2], Endang Darmawan[3] and Akrom Akrom[4,5]*

[1] Department of Public Health and Family medicine, Faculty of Medicine and Health Science, Universitas Muhammadiyah Yogyakarta, Yogyakarta, Indonesia; hidayatifkumy@yahoo.co.id
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Abstract: Background: Traditionally, Curcuma xanthorriza (CX), black cumin seed (BC), and honey have been used by the Indonesian people as medicinal ingredients to treat various health symptoms. CX extracts and BC have been proven in the laboratory as chemopreventive agents, antioxidants, and immunomodulators. In this study, we developed CX extract, BC oil, and honey into herbal honey preparations (CXBCH) and hypothesized that the preparations show chemopreventive activity. The purpose of the study was to determine the CXBCH potential as chemopreventive, antioxidant, and immunomodulatory. Method: In this experimental laboratory research, antioxidant, immunomodulatory, and cytotoxic activities were tested on human mammary cancer cell line (T47D cells) while the chemopreventive activity of the CXBCH preparations on Sprague Dawley (SD) rats induced with dimethylbenzene(a)anthracene (DMBA). Result: CXBCH preparations demonstrated immunomodulatory, antioxidant, and cytotoxic activities in T47D, Hela, and HTB-183 cells and in DMBA-induced SD rats, as the preparations inhibited tumor nodule formation, increased the number of CD4, CD8 and CD4CD25 cells, and glutathione-S-transferase (GST) activity, and decreased serum NO levels. Conclusion: CXBCH preparations display chemopreventive, antioxidant, and immunomodulatory properties.

Keywords: herbal honey; Curcuma xanthorriza; Black cumin seed; chemopreventive; antioxidants; immunomodulatory

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1. Introduction

Polycyclic aromatic hydrocarbons (PAH) 7,12 dimethylbenzene(a)anthracene (DMBA) is an air pollutant [1]. PAH compounds are one of the triggers for the increase in the incidence of breast cancer[2], lung cancer[3], and skin cancer[4]. Cigarette smoke and vehicle engine fumes are the primary sources of PAHs. Currently, smokers in Indonesia are estimated at more than 33% [5]. Indonesian urban communities are estimated to breathe polluted air daily with PAH content equivalent to PAH content in 7 cigarettes, even though they are not active smokers [6]. The content of PAH compounds in smokeless tobacco ranges from 0.1-90 ng/g [7][8], meanwhile PAH has been found in high

concentration in tobacco cigarettes (40-100 ng per cigarette) [9][10][11]. Furthermore, it has been established that cigarettes contain 30-80 ng of benzanthracene molecules. whereas it ranges from 25 to 280 ng for every gram of motor vehicle dust[12]. According to research conducted in a Japanese city, there are 0-83 picograms of benzanthracene compounds and.11–1923 picograms of total PAHs in per cubic meter of air[13][14]. Although there hasn't been any research on this subject up to this point, it is assumed that Indonesia has significant amounts of PAHs and benzanthracene in its urban air due to the country's high population of smokers and motorized vehicles[15].

DMBA is converted to DMBA-3,4-diol-1,2-epoxide by cytochrome P450 enzymes 1A1 or 1B1 (CYP1A1 or CYP1B1) and microsomal hydrolase enzymes (DMBA-DE)[16]. Microsomal epoxide hydrolase (mEH) converts DMBA-3,4-epoxide to DMBA-3,4-dihydrodiol (DMBA-3,4-diol); subsequently, CYP1A1 or CYP1B1 oxidizes DMBA-3,4-diol to its ultimate carcinogenic form, DMBA-3,4-diol-1,2-epoxide[17]. DMBA-DE is a genotoxic[18] and immunosuppressive active metabolite of DMBA[19]. DMBA exposure and cigarette smoke are associated with increased serum nitric oxide (NO) levels[20]. In physiological processes including cell signaling and inflammatory management, nitric oxide (NO) molecules are vital [21]. At the same time, NO in excessive amounts will be oxidative reactive, and genotoxic[22]. DMBA exposure is also associated with decreased immune response[23]. Administration for five days of DMBA 50 and 150 mg/kg BW in C57BL/6N wild type (WT) mice caused a decrease in spleen weight, lymphocyte count, and T lymphocyte proliferative activity, and suppressed bone marrow activity. Spleen weight, total lymphocyte count, and T lymphocyte proliferative activity were inversely related to the levels of DMBA metabolites in spleen tissues. DMBA levels in the blood are associated with the suppression of lymphoid tissues (colony forming unit (CFU)-preB) and myeloid tissues (CFU-GM) [16] [24]. DMBA levels also reduce the number of bone marrow lymphoid cells and blood lymphocytes[16]. DMBA induction inhibits iNOS expression, NO secretion, and IL-12 secretion by macrophages[25]. CD4 and CD8 T lymphocytes maintain adaptive cellular immune responses against neoplasms [26]. DMBA induction has decreased the number of lymphocytes and the immune response[17]. The decrease in the number of lymphocytes and the immune response is thought to be one of the mechanisms for the development of genetic stress into neoplasms and neoplasms into tumor tissues or cancer[27]. More people, including children and pregnant women, are exposed to carcinogenic compounds, as the number of smokers and PAH pollutants in the air rise with the growing number of motor vehicles. This condition has increased the incidence of cancer in Indonesia [6][28].

The elimination of DMBA-DE from the body is accelerated in the presence of the antioxidant enzyme glutathione-S-transferase (GST)[29]. The GST enzyme conjugates with DMBA-DE into a water-soluble compound that makes it easy to be excreted[30]. Accelerated elimination of DMBA-DE is associated with detoxification, immunoprotection, and inhibition of carcinogenesis[31]. Antioxidant agents and herbal immunostimulants are believed to be able to inhibit the conversion of DMBA into DMBA-DE, and the decrease in lymphocytes and immunological responses due to DMBA exposure are believed to be effective in avoiding carcinogenesis [32]. Curcuma xanthorriza (CX) and black cumin seed (BC) (Nigella sativa) have been shown to exhibit immunomodulatory, antioxidant, and chemopreventive properties [33][34]. Researchers have demonstrated the BC chemopreventive activity and thymoquinone via cytoprotective antioxidant effects by inhibiting the CYP gene (phase I) activity and increasing the GST gene (phase II) activity via the activation of Nrf2, thereby increasing the production of the GST enzyme[34]. The administration of thymoquinone at 50 mg/kg BW has been shown to increase the antioxidant capacity of test animals, as indicated by an increase in SOD enzyme levels and a decrease in lipid peroxide [26][27][35]. Thymoquinone can also act as a scavenger agent and neutralize free radicals to reduce DMBA-DE levels[36]. Thymoquinone has been proven to decrease DNA adduct formation and nodule formation in DMBA-induced SD rats[37]. Like BC, empirically, Curcuma xanthorriza (CX) has also

been used by the people of Indonesia as an immune system booster[33], anti-inflammatory[38], and antioxidant [39]. Indonesian people traditionally use CX on children with eating difficulty as it can serve as an appetite enhancer [33]. The main active substances of CX are Xanthorrhizol, curcumin, and curcuminoids [40]. Xanthorrhizol has been shown to display antioxidant activity by suppressing lipid peroxidation and decreasing Reactive Oxygen Superfamily (ROS) production [41]. Curcumin, one of the main active ingredients of CX, has been shown to have anti-inflammatory effects, be safe to use, and be well tolerated [42]. Honey contains various active substances, and its sweet taste has been used as a mixture in various traditional medicines to enhance the taste[43][44]. CX and BC combination with the addition of honey is thought to have a synergistic effect, by increasing antioxidant and immunomodulatory activity[45]. The research team has developed herbal honey preparations consisting of black cumin seed and curcuma xanthorriza with honey as the solvent called "CXBC herbal honey preparations" or CXBCH. It is hypothesized that CXBCH have chemopreventive potential due to their antioxidant and immunomodulatory properties. This study aimed to determine the chemopreventive activity of CXBCH preparations in Sprague Dawley (SD) rats induced with DMBA.

2. Materials and Methods

2.1. Instruments and Materials

2.1.1. Research Protocol, Test Materials, Positive Control, and Carcinogen

The research protocol was ethically reviewed and had received a clearance label from the research ethics committee of the Ahmad Dahlan university (Number: 012204031). A mixture of honey, black cumin seed oil, and Curcuma xanthorriza (CX) extracts (CXE) were provided by a traditional herbal medicine industry certified by the Indonesian Food and Drug Supervisory Agency. We obtained Tragacanth (Sigma-Aldrich, cat: G1128) as an emulsifier in the manufacture of this herbal honey preparation from an authorized agent of Sigma – Aldrich in Yogyakarta, Indonesia. The test plants were determined by experts (Prof.Dr. Subagus Wahyuono, Apt) from the Department of Biology Pharmacy, Faculty of Pharmacy, Gadjah Mada University. The extraction and preparation of the test materials were carried out with qualitative and quantitative phytochemical analysis. The thymoquinone (2-isopropyl-5 methyl-1,4-benzoquinone) (Sigma-Aldrich, cat. 274666-5G) and tamoxifen citrate (Sigma-Aldrich, cat.:T0015000) used as the positive control was obtained from Sigma while the carcinogen used was 7,12 dimethylbenzene(a)anthracene (DMBA) (sigma, cat. D3254), dissolved in corn oil at 100 mg/kg BW[46].

2.1.2. Experimental Cells and Animals

A viability test was performed on cancer cells (T47D, Hela and HTB-183) [47][48] while the chemopreventive effectiveness test of CXBCH preparations was carried out on female Sprague Dawley (SD) rats. We obtained cancer cell line (T47D, Hela and HTB-183 cells) from the Cancer Chemoprevention Research center (CCRC), Gadjah Mada University, Yogyakarta, Indonesia. We used 80 female Sprague Dawley rats aged 24 – 30 days with an average weight of 80-120 g obtained from the Preclinical Experimental and Animal Development Unit (PEADU), Gadjah Mada University, Yogyakarta, Indonesia. PEADU is a unit providing experimental animals from Gadjah Mada University. Before being used for the experiment, the animals were acclimatized to the experimental room and cages and grouped by the treatment they received. Food and drink were provided ad libitum.

We conducted a chemopreventive test of the DMBA chemical carcinogen model using female test animals in line with past findings. It has been demonstrated that DMBA causes cancer in female Sprague Dawley rats between four and six weeks of age[49][46]. The animals were kept in standardized climatic settings (22–28°C, 60–70% relative hu-

midity, and a 12-hour cycle of darkness and light). They were kept in properly ventilated cages and given access to unlimited amounts of water as well as pelleted food (brailer-II, Japfa Comfeed Ltd). All animal experiments were conducted in accordance with the guidelines established by Universitas Ahmad Dahlan's ethical research committee.

We provide standard feed to all groups of test animals. Japfa Comfeed Ltd.'s standard feed was ordered. Rats are often fed on brailler-II pellets (BR-II), which are made from a combination of corn, soybean meal, wheat germ, coconut meal, fish meal, meat meal, rice flour, tapioca, and premixes of coconut oil and fish oil.

2.1.3. Instruments and Materials

Equipment for phytochemical analysis and examination of the active substance content of CXBCH preparations included analytical balance (Ohaus Indonesia, type: EX224/AD), blender (Cosmos Indonesia, type:CB-812 G), 250 ml measuring cup (Iwaki Pyrex), 100 ml glass beaker (Iwaki Pyrex), electric stirrer (K-Ika), glass stirrer, Buchner funnel (Sigma-Aldrich Indonesia), compressor, porcelain cup, fridge, filter paper, glass jar, and water bath, macerator pot, steam distillation set, Spectrophotometer UV-Vis (Hitachi high-tech Indonesia), Spectra Max M5 microplate reader (Molecular Devices LLC), GCMS (Shimadzu, type:QP201SE) and TLC (Merck). The quantities of thymoquinone, curcumin, polyphenols, and flavonoids in CX extract (CXE), BC extract (BCE), and CXBCH preparations were determined using thymoquinone (Sigma; cat. 274666-5G), curcumin (Sigma-Aldrich, cat:C1386), gallic acid (Sigma-Aldrich, cat:398225), and rutin (Sigma-Aldrich, cat:R 5143) standards.

Equipment and materials for the chemopreventive test included rat cages containing husks, feed and drinking bowls, corn oil, CXBCH preparations, DMBA (Sigma, cat. D3254), distilled water, and nasogastric (NG) tube for DMBA treatment and induction. We also used a set of surgical instruments for organ harvesting, gloves, sterile disposable syringes, microspuit injector, glassware, mixer, vacutainer with anticoagulant, microtome, microscope, equipment for narcotics, ether or chloroform, 10% formalin as an organ fixation solution, and Hematoxylin and Eosin as coloring dyes for histopathological tests.

Materials and tools for antioxidant enzyme testing included Glutathione s – transferase assay kit (Cayman chemical company, catalog 703302); GST assay buffer (cat. 703310), GST sample buffer (cat. 703312), GST assay (control) (cat. 703314), GST glutathione (cat. 703316), GST CDNB (1-Chloro-2-4-dinitrobenzene) (cat. 703318), 96 well plate (colorimetric assay) (cat. 400014), 96 well cover sheet (cat. 400012); phosphate buffer (K_3PO_4); PBS (phosphate buffered saline); RPMI; centrifuge "Sovval Biofuge primo R," micro pipette and glassware. Griess A and B solutions for measuring NO levels were also used.

Instruments and materials for testing the immune response included a centrifuge (Sovval Biofuge primo R), 5-1000µL micropipette with disposable tips, beakers, flasks, disposable tips, 5-10 ml pipettes, glassware, and plates with 24 wells and 96 wells. Solutions for cell culture included Tris Buffered Ammonium chloride (TBAH) and FBS (Fetal bovine serum) (Biochrom, Berlin, Germany); L-glutamine 200mM (100x) (Invitrogen, Paisley, UK); amino acids solution (50x) (Invitrogen, Paisley, UK); Penicillin-streptomycin-solution (Invitrogen, Paisley, UK); Trypsin-EDTA (1x) (Invitrogen, Paisley, UK); Corn oil/corn oil; distilled water; sodium nitrate; trypan blue; PBS (phosphate buffered saline), DMEM and RPMI growth media. Flow cytometry required the "Sovval Biofuge primo R" centrifuge, 5-1000L micropipettes with disposable tips, beakers, flasks, disposable tips, 5-10 ml pipettes, glassware, 15 and 50 L falcon tubes, vortex mixer and CO_2 incubator, flowcytometer; freezer; eBioscience® Flow Cytometry Staining Buffer (eBioscience Cat. No. 00-4222), and tritest reagent.

2.2. Preparation of CXBC Herbal Honey, Phytochemical Analysis, and Active Substance Content Testing

The CXBCH preparations were formulated from a mixture of CX extract, BC extract, and honey and prepared by CV Al Afiat, a small traditional medicine business certified by the Food and Drug Supervisory Agency (FDSA) of the Republic of Indonesia. In brief, the preparation process was as follows: extracts of CX and BC were weighted according to formulation composition and then mixed with honey. The mixture was then stirred and heated at a temperature of 30-40°C and at a constant speed so that it was evenly mixed into CXBCH preparations.

As other researchers have previously done, phytochemical analysis was performed on CX extract, BC extract, and CXBCH preparations to determine the qualitative content of alkaloids, polyphenols, flavonoids, saponins, and triterpenes. Thin layer chromatography was used to perform preliminary phytochemical investigation on CXE, BCE, and CXBCH preparations. TLC was performed on alu-minium-backed silica gel plates (Merck, Darmstadt, Germany, Art. 5533). The plates were heated at 103 C for 3–4 minutes before being exposed to UV light (254 nm) or being sprayed with anisaldehyde-sulfuric acid reagent (anisaldehyde 0.5 mL, glacial acetic acid 10 mL, methanol 85 mL, conc. sulphuric acid 4.5 mL). As indicated by earlier researchers, we also used GC-MS and LC-HRMS analysis to identify the bioactive compounds in the CXBCH[50].

Folin-Ciocalteau reagent (Merck, Germany) and standard gallic acid (Sigma-Aldrich, cat:398225) were used to check the total phenol content. The calibration curve was prepared by mixing 90 µL of Folin-Ciocalteau reagent and 90 µL of NaCO3 solution with gallic acid. 10 mg of the sample was weighted, dissolved in 10 mL of ethanol, and homogenized. After filtering the material, the filtrate was used for analysis. Five hundred microliters of filtrate, 7.5 milliliters of distilled water, and 500 microliters of Folin-Ciocalteu reagent were to be pipetted. After being homogenized and incubated for 8 minutes, the samples were analyzed. After incubation, 1.5 mL of sodium carbonate solution with a 20% concentration was added. After another incubation for 1 hour, the absorbance was measured at a wavelength of 765 nm, and the concentration of polyphenols in the sample was calculated[51].

Rutin standards were used to measure the total flavonoid content using the Aluminum Chloride Colorimetric technique. Rutin (mg) equivalent was used to represent the total flavonoid concentration per gram of sample (1000 ppm). We make a 5% concentration stock sample solution (50000 ppm). A 10 mL measuring flask containing .1 mL of the stock sample solution was pipetted into, filled with distilled water to the mark, and homogenized to create a sample solution with a 5000ppm concentration. Before vortexing, pipette 500 µL of the 5000ppm sample solution and 100 µL of the 10% AlCl3 reagent solution. The preparation was then mixed with 100 µL of 1 M sodium acetate reagent, vortexed, and finally, 2.8 mL of distilled water was added before the absorbance was calculated[52].

The thymoquinone levels were measured with UV-vis spectrophotometry with the same procedure as carried out by previous researchers. As much as 12.5 mg of thymoquinone was weighted and then dissolved in methanol to a final volume of 25 ml to obtain a concentration of 500 µg/mL. The thymoquinone level calibration curve was generated by diluting the 500 µg/mL concentration into five different thymoquinone concentrations. A 0.1 mL thymoquinone mother liquor was pipetted into a 25 mL volumetric flask, dissolved in methanol to a final volume of 25 mL, and then quantified using a UV-Visible spectrophotometer at a wavelength of 200 – 400 nm to find out the specific wave length number of thymoquinone. The thymoquinone levels in CX extract, BC extract, and CXBCH preparations were carried out as follows: the sample was prepared by weighting a certain amount of BC extract and then dissolved in methanol to a final volume of 10 mL. The test material was then homogenized with a vortex for 2 minutes. After being allowed to stand for 1 minute, the methanol layer at the top was taken out. After that, the sample was filtered using a 0.45 m syringe filter and then injected into

High-Performance Liquid Chromatography (HPLC) with an injection volume of 20 µL, and the peak area was seen. The peak area obtained was then substituted into the regression equation on the calibration curve as the Y value to obtain the sample concentration in ppm. The % w/w concentration was calculated [53].

The chromatographic method was used to determine the curcumin levels in CXBCH preparations. We started the process by determining the wavelength and calibration curve by analyzing the standard solution with a UV-Vis chromatography instrument three times. The average results of the measurements were used as a reference in the analysis of curcumin with HPLC. Calibration curves were made using a series of reference standard solutions with five different concentrations (0.5, 1, 2, 5, and 10 µg/mL). HPLC conditions were column E-C18, column temperature of 40◦C, mobile phase mixture of acetonitrile and acetic acid 2% (55:45), and flow rate of 0.5 mL/minute with UV-Vis detector at a specified wavelength. Curcumin content was determined by entering the average value of the sample area from three replications into the linear regression equation from the standard curve so that the content was obtained in g/mL units. The results obtained were then converted into ppm units. Each prepared sample was put in ultrasonic degassing to remove air bubbles. The sample was then filtered with a 0.45 m syringe filter, injected into the HPLC system at 10.0 µg/mL, and replicated three times[54].

At the National Research and Innovation Agency, Yogyakarta, Indonesia, the LC-HRMS analysis of ethyl acetate and the aqueous fraction was carried out using an Thermo scientific The Vanquish HPLC coloumb Acclaim PM 100 C18 3 µmx150mm-Q Exactive Orbitrap HRMS. Gas temp: 30°C, gas flow: 11.01/min, nebulizer: 40 psi, VCap: 3500, fragmentor: 175, skimmer 1: 65.0, and octupole RF Peak: 750 are the settings for the source and scan parameters. Acetonitrile, a 5 mM acetate buffer, and water are used to elute the solvent at a flow rate of 1.5 mL/min. Starting with 5% acetonitrile for 0.1 minutes, the elution gradient was increased to 30% acetonitrile for 10 minutes, 80% acetonitrile for 32 minutes, and then returned to its initial settings. The column temperature was maintained at 30°C throughout the entire procedure. The flow cell of the diode array detector was traversed before the column elute was sent to a Q-TOF HRMS equipped with an electrospray interface. With a scan rate of 1.03 and a mass range of 100-2000 daltons, positive electron spray ionization (ESI-positive mode) was used to analyze the mass spectrum[55].

2.3. Reactive Radical Binding Activity Test of CXBCH Preparations

CX extract (CXE), BC extract (BCE), and CXBCH preparations were tested for reactive radical binding activity. Samples were made of 100 ppm mother liquor by dissolving 10 mg of extract in 100 ml of methanol PA. Furthermore, dilution using methanol pa solvent was done with varying concentrations of 5 ppm, 6 ppm, 7 ppm, 8 ppm, and 9 ppm. DPPH stock solution was prepared by dissolving 5 mg of solid DPPH into 100 ml of methanol PA. Then a comparison solution was prepared: a control solution containing 2 ml of methanol PA and 1 ml of 50 ppm DPPH solution. Every 2 ml of sample solution and 2 ml of DPPH solution were prepared for the test sample. Then, it was incubated for 30 minutes at 27°C until there was a color change from DPPH activity. All the samples were made in triplicate. Samples of the extracts and CXBCH preparations that had been incubated were then tested for absorbance values using a UV-vis spectrophotometer at a wavelength of 517 nm. The IC50 value is the sample concentration required to scavenge 50% of DPPH free radicals, which we calculated by plotting the percent inhibition against the log sample extract concentration[56].

2.4. Viability Test

Cytotoxicity activity of CXBCH preparation was tested out on T47D, Hela, and HTB-183 cells. Cancer cells were cultured in DMEM containing 10% FBS supplemented with 1% penicillin (100 units/mL) and streptomycin (100 µg/mL) at 37 °C, 5% CO_2 in an incubator. The test material's cytotoxicity activity was determined using the 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2-H-tetrazolium bromide (MTT) assay method. Briefly, T47D, Hela and HTB-183 cells were plated in 96-well plates (5 × 103 cells/well) in DMEM containing 10 % FBS and 24 h later they were treated with CXBCH (0, 125, 250, 500, 10000 µg/mL) and incubated for 72 h in DMEM with 1% FBS. Then MTT reagent (0.2 mg/mL) was added to each well and incubated for 4 hours. A 200 µL of dimethyl sulfoxide (DMSO) reagent was added to dissolve the formazan product in each well, followed by measuring the absorbance at a wavelength of 595 nm using a spectrophotometer (SpectraMAX M5, Molecular Devices, CA)[57].

2.5. Chemopreventive Testing in Animal Models of Cancer

2.5.1. *DMBA Induction and CXBCH Administration in Experimental Animals*

After undergoing quarantine for one week, a total of 80 Sprague Dawley (SD) rats aged four weeks were then randomly grouped into eight groups of ten rats. Group I was the normal group where female SD rats received standard food and drink. Group II was given CXBCH1 (equivalent to 1x5 ml/70kg BW), Group III CXBCH2 (equivalent to 2x5 ml/70kg BW), Group IV CXBC3 (3x5 ml/70 kg BW) as the treatment groups. Because each set of test animals weighed 100 grams, a 1 mL solution comprising 0.09 mL of CXBCH was administered along with volumes of 1/2 mL, 1 mL, and 1.5 mL of CXBCH1, CXBCH2, and CXBCH3. Group V, as the positive control group, received thymoquinone orally at 20 mg/kg BW [40]. Group VI, as the positive control group, was given tamoxifen 0.6 mg/kg BW/day [41]. CXBCH, thymoquinone and tamoxifen were administered for five weeks during DMBA induction followed by four weeks post-induction. As the negative control group, Group VII received DMBA 2x20 mg/kg BW/week for five weeks and standard food and drink [11]. Group VIII was the solvent control group, where the test animals received standard food, drink, and solvent (corn oil). Each SD rat was given a maximum volume of 2 ml that contained the active ingredients according to the dose[35].

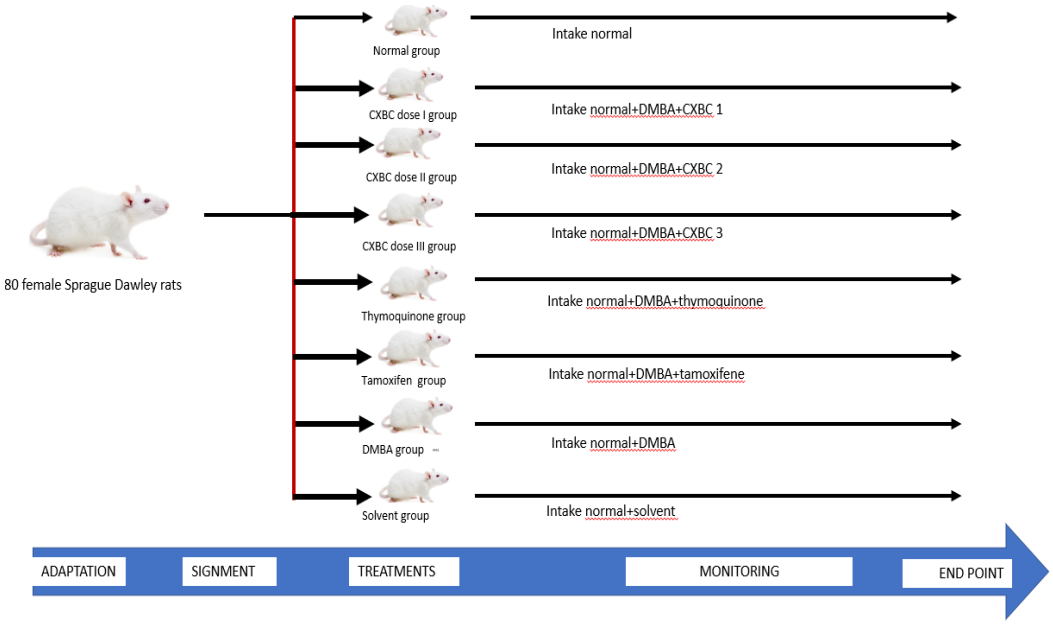


Figure 1. Placement and treatment of test animals.

All groups, except the normal and solvent control groups, were induced with DMBA. Carcinogenesis experiments used the carcinogen DMBA 20 mg/kg BW, which

was administered intragastrically with a probe twice a week for five weeks. Observations on tumor formation started from the last DMBA administration until the 30th week of treatment.

2.5.2. *Examination Procedure of the Chemopreventive Effect of CXBCH preparations*

2.5.2.1. *Observation of Clinical Manifestations and Nodule Formation of*

Examination of the main clinical manifestations was carried out on body weight, survival, and biochemical features for the physiology of the kidney, liver, and peripheral blood. Body weight measurements of each rat were carried out twice a week. Peripheral blood and blood chemistry examinations were carried out at the Integrated Testing and Examination Institute unit I. Peripheral blood examinations were carried out using a Sysmex KX-21 hematology analyzer (Sysmex inc), while blood chemistry examinations (SGPT, SGOT, urea, and creatinine) using a spectrophotometric device (Microlab 3000). Blood samples were taken from the rat through the orbital sinus as much as ± 1.5 ml. Blood from the orbital vein was collected in a labeled Eppendorf tube containing an anticoagulant and then divided into two: one part for examination of peripheral blood images and another part for blood biochemical examination. The blood for blood biochemical examination was allowed to stand for 15 minutes, before being centrifuged at 4000 rpm for 10 minutes (1,789 G), and then the supernatant (serum) was taken and used to determine SGPT, SGOT, urea, and creatinine levels [42][35].

2.5.2.2. Examination of Nodule Incidence and Multiplication

The antitumorigenic activity of the test materials was observed clinically, macroscopically, and microscopically. The macroscopic observation was carried out by palpating the mammary organs, measuring the formation of tumor nodules (incidence), and counting the number of nodules formed (nodule multiplicity) in the breast tissues. The day or date when the tumor nodule was first seen or felt and the number of tumor nodules were recorded accordingly. Observation of the tumor nodules was carried out after the DMBA administration was complete, starting from the eighth week of the experiment by observing and palpating. The presence of new nodules in the mammary organs was counted as the incidence of tumor nodules. The chemopreventive effect of the CXBCH preparations was expressed by (i). the incidence of nodule formation between the treatment groups and the DMBA group; (ii). The number of tumor nodules per group and tumor multiplicity; and (iii). Time of nodule formation [43][58].

2.5.2.3. Histopathological Examination

Histopathological examination was carried out at the 30th week of the experiment to determine changes in the structure of tissues and cells in the test animals' mammaries. Organ harvesting was carried out as follows: rats were sacrificed, and their stomach skins were cut. The rats were sacrificed using chloroform vapor. The tumor nodules or mammary tissues that needed to be examined were removed, and then cleaned with 0.9% of NaCl solution, and put in a pot containing 10% formalin. Technicians made histopathological preparations at the Anatomical Pathology Laboratory, Faculty of Medicine, Universitas Gadjah Mada (UGM), Yogyakarta. The fixation process was carried out on the mammary organs. After the fixation process, trimming or thin cutting of tissue approximately 4mm thick was carried out using a scalpel knife No. 22-24. The tissue was loaded in an embedding cassette which functioned as a network holder.

Tissue dehydration was carried out in a tissue processor after trimming using the dehydrating liquid, ethanol, to remove water contained in the tissue. This dehydrating liquid was then cleaned with a cleaning reagent, namely toluene, which would be replaced with paraffin by penetrating the tissue. This process is called impregnation. The tissue was put in hot paraffin, which would infiltrate the tissue. This process is intended

to make it easier to cut the tissue using a microtome. The tissue was then cut using a microtome knife with a thickness of 5µm. The layer was then placed on a slide to color. Staining was performed using Hematoxylin and Eosin. After the tissue on the slide was stained, mounting was carried out by dripping the mounting material and covering it with a cover glass. After the preparations have finished, tissue inspection and shooting were carried out at the Pathology Laboratory of the Faculty of Veterinary Medicine, Gadjah Mada University (UGM). Examination of the tissue using a light microscope was carried out by an Anatomical Pathologist from the Faculty of Veterinary Medicine, Gadjah Mada University, and the photography was also carried out at the same laboratory. Microscopic observations included the histological condition of the mammary gland organs from the H&E staining. H&E preparations were observed descriptively to determine the carcinogenesis stages. Upon microscopic observations based on the proliferative level of epithelial cells, the tissue was categorized as normal, hyperplasia/dysplasia, or adenocarcinoma according to the histopathological appearance [44][59].

2.6. CXBC Antioxidant Activity Examination Procedure

2.6.1. Examination of Serum NO levels

Examination of serum NO levels was carried out using the colorimetric method on the blood samples taken through the orbital vein. As much as two cc of blood was put into a blood collection tube, and a colorimetric determination of nitric oxide levels was carried out using Griess's solution [45][60].

2.6.2. Liver and Spleen GST Enzyme Activity

As previously investigated, GST enzyme activity was determined by enzymatic analysis [46]. SD mice that had been treated for seven weeks and induced with DMBA at the end of the 30th week, the day before data collection, fasted for 24 hours. Then, the test animals were decapitated, their liver and spleen tissues were removed, and samples were made. As much as 1 gram of liver or spleen tissue was taken from the cytosolic fraction of the liver microsomal to measure the total GST enzyme activity. Then, the samples were washed with PBS. After being considered clean, the tissue was homogenized in 5 -10 ml of cold buffer (100 mM K3PO4, pH 7.0, containing two mM EDTA) and centrifuged at 10,000 g for 15 minutes at 4◦C. The liver homogenate supernatant obtained was then examined for the GST enzyme activity using the GST ELISA kit following the industry standard procedure. The speed of GST enzyme activity was determined based on the formation of GSH conjugation with 1-chloro-2-4-dinitrobenzene (CDNB). The final assay volume was set at 200 µl per well.

The room temperature for the test was 25◦C. GST activity check steps were carried out according to the standard instructions from the industry. Each well was filled with 150 µL of assay buffer, 20 µL of glutathione, and 20 µL of the sample. The reaction was initiated by quickly adding ten µLCDNB to each well. Then, the microplate was shaken for a few seconds to corrode the test material. The reaction results were read every minute (at least 5 x) with an Elisa reader at a wavelength of 340 nm. The GST reaction rate on an ELISA reader at a wavelength of 340nm can be determined using the CDNB extinction coefficient of 0.00503 µM-1. At 25°C every minute, 1 unit of enzyme will conjugate with one nmol of CDNB by reducing glutathione. GST activity was calculated using the following formula:

$$\text{GST activity} = \frac{\Delta A340/\text{min} \times 0.2\,\text{ml} \times \text{sample dilution}}{0.00503\,\mu\text{M-1} \times 0.02\text{ml}}$$

2.7. Monitoring the Immune Response

2.7.1. Number and Types of CD4, CD8 and CD4CD25 Lymphocytes by Flowcytometer

Examination of the number and types of leukocytes was carried out using a Sysmeix kx 12 hematology analyzer in the Integrated Research and Testing Laboratory (IRTL),

Gadjah Mada University. We examined the number of CD4, CD8, CD4CD25, and CD8CD25 by flow cytometry in the Clinical Pathology Laboratory, Gadjah Mada University [47][61].

Blood that had been collected in a vacutainer tube containing an anticoagulant was then examined with a flow cytometer with the following procedure: (i). As much as 50 µL of the test material/specimen was pipetted into a falcon tube; (ii). A total of 10 pL of the CD4/CD8 FITC/CD25 tritest reagent per CP was added to each falcon tube that had been filled with the test material; (iii). The specimens and tritest reagents in the Falcon tube were mixed until homogeneous with a vortex mixer, then incubated for 15 minutes at 20-25°C and dark room; (iv). While waiting for incubation, the FACS reagent was diluted, where 50 pL of FACS solution was diluted 10x by adding 450 pL of distilled water, then mixed until homogeneous; (v). After the incubation time was complete, the sample was added with 450 µl of the already diluted FACS reagent (lx);. (vi). After adding the FACS reagent to each falcon tube, the sample was mixed until homogeneous with a vortex mixer, then incubated for 15 minutes, at temperature 20-25°C, in a dark room; (vii). After the incubation was over, analysis was performed using BD Biosciences FACS and CellQuest software to determine CD4/CD8/CD25 counts.

2.6. Data Analysis

The bioactive content of TLC results and measurements of total flavonoids, polyphenols, curcumin and thymoquinone are presented descriptively.Raw data files acquired from the LC-HRMS were processed using MZmine 2 and then Mestre Nova 12.0 for compound annotation using PubChem, Dictionary of Natural Products 2, ChemSpider, and METLIN database.

Using one-way ANOVA, the test findings of cell viability and antioxidant capacity were compared between groups for various means.

The repeated measure method was used to assess data on body weight development. Using one way ANOVA and post hoc analysis, the mean differences between groups were examined for the blood cell count, SGPT, SGOT, serum urea, and creatinine.

The number of nodules and nodule weight were then given in a descriptive manner after the tumor incidence was expressed as a percentage of the tumor occurrence in each group. By characterizing cell proliferation activity, metaplasia, mutations or neoplasms, and neoplasms progress, the results of histopathological observations of tumor nodules were assessed descriptively and qualitatively. If there was no change in the proliferation, it was expressed as normal proliferation, and if there was an increase in activity, it was expressed as hyperproliferation.

The mean differences between groups of GST, NO levels and CD4, CD8, and CD4CD25 cell counts were examined by one way ANOVA. The data was checked for normality with Kolmogorov Smirnov and deemed to be normally distributed before the mean difference test with one way ANOVA. A 95% confidence level was used for all statistical tests.

3. Results

3.1. Active Substance Content of CXBCH preparations

The active substance contents of CXBCH preparations were tested qualitatively and quantitatively.

Thin layer chromatography was used to qualitatively assess the active ingredient of CXBCH, CXE and BCE. Table shows the findings of the qualitative and quantitative analysis of the active substance content.

Table 1. Phytochemical analysis of CXE, BCE, and CXBCH preparations.

Test	Samples and materials		
	CXE	BCE	CXBCH Preparation

Qualitative			
Alkaloids	++	+	++
Flavonoids	++	++	++
Phenolic	++	++	++
Saponins	+	+	+
Triterpenoids	+	++	+
Quantitative			
Polyphenol	142.23 ppm	42.51ppm	38.87 ppm
Flavonoids	116.41 ppm	31.74 ppm	56.86 ppm
Thymoquinone	-	54.71 mg/g	46.45 mg/mL
Curcumin	62.28 mg/mL	-	68.86 mg/mL

The results of the phytochemical analysis (table 1) show that the CXBCH preparations qualitatively contained alkaloids, flavonoids, phenolics, saponins, and triterpe-noids. It is known quantitatively that the CXBCH preparation contains 38.87 ppm of polyphenols, 56.86 ppm of flavonoids, 46.45 mg/mL of thymoquinone, and 68.86 mg/mL of curcumin.

Bioactive Compound profile on CXBC with GCMS and LC-HRMS

We have observed the volatile compound in the CXBCH preparation using GCMS. The figure and table shows the findings of the profile of the volatile compound on CXBCH.

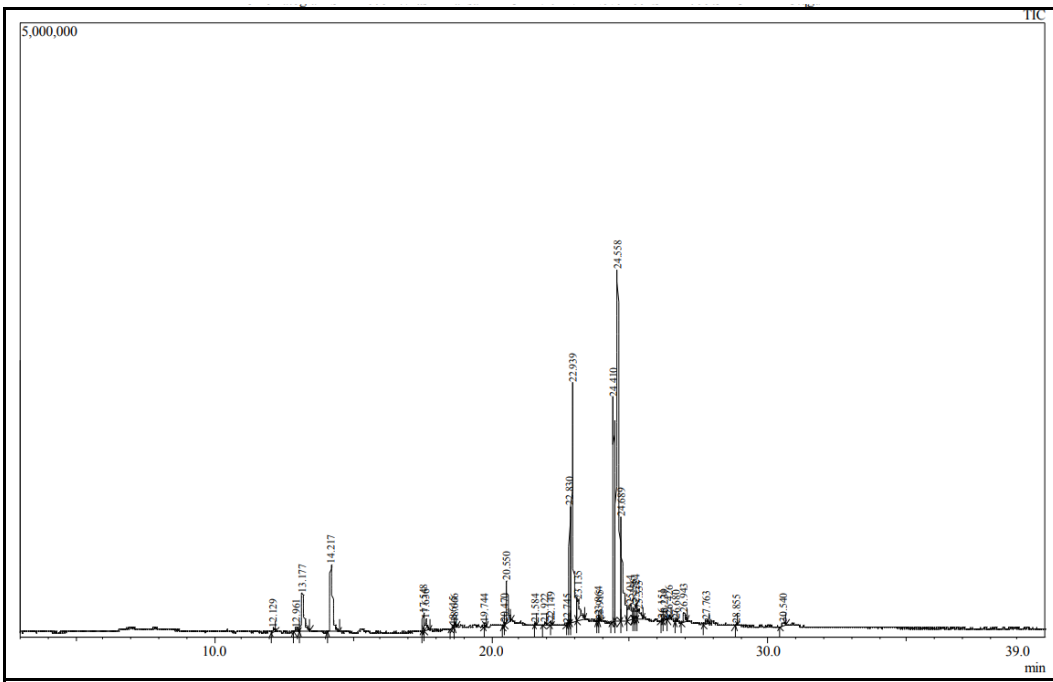


Fig 2. GCMS analysis of CXBCH preparations

Observations using the GCMS tool We obtained data for more than 30 volatile compounds from CXBCH preparations. The major compounds present in the CXBCH were 9-Hexadecenoic acid (33.65%), Hexadecanoic acid (16.49%), Ethyl linoleate (10.99%), octadecanoic acid (8.88%), gamma.-curcumene (6.82%), benzene, 1-(1,5-dimethyl-4-hexenyl)-4-methyl (4.14%), and Hexadecanoic acid, ethyl ester (3.36%). The entire list of compounds observed with GCMS is presented in Table.

508
509
510

Table 2. profile volatile Compounds of CXBCH preparations, CXE, and BCE from the GCMS examination

511

No Peak	R.Time	I.Time	F.Time	Composition	Formula	Name
1	12.129	12.045	12.240	0.32	C15H24	1,6,10-dodecatriene, 7,11-dimethyl-3-methylene-, (e)-
2	12.961	12.870	13.080	0.32	C15H24	**gamma.-curcumene**
3	13.177	13.080	13.455	**4.14**	C15H22	**benzene, 1-(1,5-dimethyl-4-hexenyl)-4-methyl-**
4	14.217	14.090	14.495	**6.82**	C15H24	**gamma.-curcumene**
5	17.548	17.485	17.600	0.94	C15H22O	beta.-Elemenone (CAS)
6	17.636	17.600	17.765	0.76	C9H14N2O3S	n-(4-hydroxyphenyl)-n,n',n'-trimethylsulfamide
7	18.615	18.535	18.630	0.11	C15H22O2	6-(1-Hydroxymethylvinyl)-4,8a-dimethyl-3,5,6,7,8,8a-hexahydro-1H-naphthalen-2-one
8	18.666	18.630	18.750	0.27	C17H33Cl	7-Heptadecene, 1-chloro- (CAS)
9	19.744	19.710	19.780	0.12	C15H26O	Juniper camphor
10	20.470	20.415	20.505	0.10	C24H32O6	medrol acetate
11	20.550	20.505	20.705	**3.04**	C9H12O	Cc1cc(C)c(O)c(C)c1
12	21.584	21.550	21.640	0.08	C20H40O	3,7,11,15-Tetramethyl-2-hexadecen-1-ol
13	21.922	21.880	22.010	0.13	C12H22O	2-methyl-10-undecenal
14	22.149	22.110	22.205	0.12	C21H38O4	9-Octadecenoic acid, 12-(acetyloxy)-, methyl ester, [R-(Z)]- (CAS)
15	22.745	22.695	22.785	0.08	C12H22O2	cyclooctancarbonic acid, 4-methyl-, ethylester
16	22.830	22.785	22.860	**3.36**	**C18H36O2**	Hexadecanoic acid, ethyl ester (CAS)
17	22.939	22.860	23.105	**16.49**	**C16H32O2**	Hexadecanoic acid (CAS)
18	23.135	23.105	23.365	**2.06**	**C20H40O2**	Eicosanoic acid (CAS)
19	23.864	23.795	23.895	0.24	C19H36O2	9-Octadecenoic acid, methyl ester (CAS)
20	23.915	23.895	23.975	0.10	C46H58N4O8	14'-epi-20'-deoxyvincovaline
21	24.410	24.345	24.465	**10.99**	**C20H36O2**	**Ethyl linoleate**
22	24.558	24.465	24.660	**33.65**	**C16H30O2**	**9-Hexadecenoic acid (CAS)**
23	24.689	24.660	24.925	**8.88**	**C18H36O2**	**octadecanoic acid**
24	25.014	24.925	25.110	**2.08**	**C22H38O2**	Cyclopropaneoctanoic acid, 2-[[2-[(2-ethylcyclopropyl)methyl]cyclopropyl]methyl]-, methyl ester (CAS)
25	25.136	25.110	25.170	0.56	C16H27NO4	6-Nitro-cylohexadecane-1,3-dione
26	25.224	25.170	25.265	**1.06**	**C18H36O3**	Hexadecanoic acid, 2-hydroxyethyl ester (CAS)
27	25.335	25.265	25.485	**1.12**	**C21H38O2**	11,14-Eicosadienoic acid, methyl ester (CAS)
28	26.151	26.110	26.200	0.10	C18H32O2	9,12-Octadecadienoic acid (Z,Z)- (CAS)
29	26.239	26.200	26.360	0.18	C18H34O2	9-Octadecenoic acid (Z)- (CAS)
30	26.426	26.360	26.505	0.27	C20H40O2	Eicosanoic acid (CAS)
31	26.680	26.640	26.720	0.10	C20H37ClO2	2-Chloroethyl oleate
32	26.943	26.885	27.055	0.69	C21H40O3	Oleic acid, 3-hydroxypropyl ester (CAS)

33	27.763	27.680	27.875	0.35	C19H38O4	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester (CAS)
34	28.855	28.780	28.895	0.09	C12H24O2	Decanoic acid, ethyl ester (CAS)
35	30.540	30.435	30.640	0.28	C20H38O3	9-Octadecenoic acid (Z)-, 2-hydroxyethyl ester (CAS)
	Total			100.00		

Based on the literature search, some compounds found in the CXBXH have been reported to display antioxidant, chemopreventive, anticancer, anti-inflammatory, immunomodulatory, antibacterial and antimicrobial activities. For example, n-hexadecanoic acid, gamma-curcumene, methyl ester eicosadienoic acid, Cyclopropaneoctanoic acid and ethyl-cyclodocosane were some of the compounds identified by GCMS (Table 3) and in CXBCH preparation. Anti-inflammatory as well as anti-cancerous compounds identified included, 9,12-octadecadienoic acid (z,z), octadecanoic acid, heptadecyl triflouroacetate and alloaromadendrene. Major compounds of CXBCH were revealed to be 6.12 9-Hexadecenoic acid (33.65%), 4.41 Hexadecanoic acid (16.49%), and 3.13 Ethyl linoleate (10.99%).

Profile of bioactive compounds in CXBCH preparations observed using Liquid Chromatography High Resolution Mass Spectrometry (LC-HRMS).

The profile of bioactive compounds in CXBCH preparations observed with LC-HRMS is presented in Figures and Tables.

Figure 3. The total ion chromatogram of the CXBCH preparation was observed using LC-HRMS

Formula, molecule name, RT, annotation delta mass and max area (absolute) observed with LC-HRMS from CXBCH preparations are presented in Table 3

Tabel 3. Formula, molecule name, RT, annotation delta mass and max area (absolute) observed with LC-HRMS from CXBCH preparations.

No	Name	Formula	Annot. DeltaMass [ppm]	Calc. MW	RT [min]	Area (Max.)
1	Linoleic acid	C18H32O2	-3.41	280.23927	22.713	73665569215
2	eremanthin	C15H18O2	-3.27	230.12993	17.869	49142859119
3	1,5-Anhydro-D-fructose	C6H10O5	-2.94	162.05235	1.57	23076928966

4	Oleic acid	C18 H34 O2	-3.18	282.25498	20.527	20748954114
5	1,5-Anhydro-6-deoxy-D-threo-hex-1-en-3-ulose	C6 H8 O4	-2.08	144.04196	1.606	16831135065
6	1-Linoleoyl glycerol	C21 H38 O4	-4.96	354.27525	21.601	16123655143
7	Monoolein	C21 H40 O4	-4.44	356.29108	23.058	15577251606
8	TURMERONE, AR-	C15 H20 O	-3.22	216.15072	17.516	10297107430
9	Meglutol	C6 H10 O5	-2.54	162.05241	3.029	10269644874
10	L-α-PALMITIN	C19 H38 O4	-4.13	330.27564	22.528	9837543783
11	9-Oxo-10(E),12(E)-octadecadienoic acid	C18 H30 O3	-3.01	294.21861	19.108	9468689328
12	(E)-6-hydroxyoctadec-4-enoic acid	C18 H34 O3	-3.02	298.24999	19.444	9449669222
13	Curcumin	C21 H20 O6	-3.55	368.12468	16.266	91424240 73
14	(E,E)-alpha-Farnesene	C15 H24	-3.33	204.18712	19.747	81766473 45
15	S-Curcumene	C15 H22	-3.69	202.1714	18.938	71516147 98
16	9(Z),11(E),13(E)-Octadecatrienoic Acid methyl ester	C19 H32 O2	-3.8	292.23912	20.093	67956163 69
17	Pyrogallol	C6 H6 O3	-1.7	126.03148	3.29	61464904 43
18	(2E)-3-(3-Hydroxyphenyl)acrylaldehyde	C9 H8 O2	-2.26	148.0521	17.861	46107429 91
19	11(Z),14(Z)-Eicosadienoic acid	C20 H36	-2.98	308.27061	21.897	45327205 59

		O2				
20	(-)-Camphor	C10 H16 O	-2.9	152.11 967	15.3 95	44824689 27
21	α-Eleostearic acid	C18 H30 O2	-2.41	278.22 391	19.1 51	40165741 14
22	NP-020521	C18 H32 O3	-2.87	296.23 429	19.1 48	39641610 53
23	Prednisone	C21 H26 O5	-4.41	358.17 645	16.4 64	34570863 74
24	(2S)-2,3-Dihydroxypropyl (11Z,14Z)-11,14-icosadienoate	C23 H42 O4	-3.37	382.30 702	19.2 52	33564977 75
25	2,2'-Methylenebis(4-methyl-6-tert-butylphenol)	C23 H32 O2	-3	340.23 921	21.2 82	28491836 80
26	Carvone	C10 H14 O	-2.35	150.10 411	18.9 21	27806658 13
27	3,4-Dihydrocadalene	C15 H20	-3.23	200.15 586	19.1 05	24077229 95
28	Oleamide	C18 H35 N O	-2.8	281.27 108	22.3 99	21728243 83
29	Monoolein	C21 H40 O4	-4.45	356.29 107	22.4 82	19827320 23
30	1-Stearoylglycerol	C21 H42 O4	-4.28	358.30 677	20.7 16	19626878 38
31	Etretinate	C23 H30 O3	-2.87	354.21 848	19.7 46	19482053 51
32	NP-019983	C20 H16 O6	-3.39	352.09 35	14.7 26	19341738 52
33	Phloroglucinol	C6 H6 O3	-1.55	126.03 15	3.55 5	19312878 84
34	Stearidonic acid	C18	-2.32	276.20	19.0	18150115

		H28 O2		829	74	51
36	geranyl quinone	C16 H20 O2	-2.98	244.14 56	17.4 68	16561239 30
37	5,6,7-Trihydroxy-8-(3-methyl-2-buten-1-yl)-4-phenyl-2H-chromen-2-one	C20 H18 O5	-2.99	338.11 441	16.2 85	15520478 92
38	5-hydroxy-4-methoxy-5,6-dihydro-2H-pyran-2-one	C6 H8 O4	-2.47	144.04 19	3.55 6	15040394 99
39	Wighteone	C20 H18 O5	-2.6	338.11 454	16.6 36	13135766 85
40	(2S)-3-(4-Hydroxyphenyl)-2-({[(3S,4S,5R)-2,3,4-trihydroxy-5-(hydroxymethyl)tetrahydro-2-furanyl]methyl}amino)propanoic acid (non-preferred name)	C15 H21 N O8	-3.71	343.12 544	1.37 4	12534772 30
41	8Z,11Z,14Z-Eicosatrienoic acid	C20 H34 O2	-3.9	306.25 469	20.6 43	12399891 32
42	(+/-)12(13)-DiHOME	C18 H34 O4	-2.72	314.24 486	18.1 38	11752509 81
43	NP-011548	C18 H34 O3	-3.44	298.24 977	20.5 63	11220642 88
44	α-Linolenic acid	C18 H30 O2	-2.95	278.22 376	21.6 67	10809060 94
45	(-)-Caryophyllene oxide	C15 H24 O	-3.36	220.18 197	14.4 71	99636505 4.1
46	Curcumene	C15 H22	-3.69	202.17 14	17.4 01	96917052 7.2
47	α-Eleostearic acid	C18 H30 O2	-2.37	278.22 392	22.4 93	87322761 1.3
48	2,4-Dimethylbenzaldehyde	C9 H10 O	-2.78	134.07 279	19.3 55	78752352 7.3
49	(3beta,24R,24'R)-fucosterol epoxide	C29 H48 O2	-3.84	428.36 379	20.3 93	59452648 4.4

50	O-Demethylcurcumin	C20 H18 O6	-2.69	354.10938	15.868	58109674.5
51	(16beta)-16,21-Epoxypregna-4,17-diene-3,11,21-trione	C21 H24 O4	-3.32	340.16633	18.569	53863935.5
52	1-[(11Z,14Z)]-icosadienoyl-sn-glycero-3-phosphocholine	C28 H54 N O7 P	-3.18	547.36205	18.924	50608138.6
53	(+/-)9-HpODE	C18 H32 O4	-3.04	312.22911	18.056	46306569.7
54	16-Hydroxyhexadecanoic acid	C16 H32 O3	-2.33	272.23451	21.832	44605729.6
55	(15Z)-9,12,13-Trihydroxy-15-octadecenoic acid	C18 H34 O5	-2.25	330.23988	16.179	43268735.6
56	5-Methoxy-7-(4-hydroxy-3-methoxyphenyl)-1-phenyl-3-heptanone	C21 H26 O4	-3.17	342.18202	19.464	43202993.4
57	3-Ethylphenol	C8 H10 O	-2.9	122.07281	16.24	38552651.2
58	1,3,7-Trihydroxy-2,8-bis(3-methyl-2-buten-1-yl)-9H-xanthen-9-one	C23 H24 O5	4.96	380.16426	22.711	36796164.2
59	1-Naphthol	C10 H8 O	-2.35	144.05718	17.615	36095297.5
60	(±)9-HpODE	C18 H32 O4	-3.04	312.22911	19.356	33950066.4
61	all-trans-4,4'-diapo-zeta-carotene	C30 H44	-2.68	404.34322	18.901	33509702.5
62	1-Methyl-4-(1-methyl-2-propenyl)-benzene	C13 H18	-1.55	174.14058	18.947	32550892.1
63	Vanillin	C8 H8 O3	-1.47	152.04712	9.205	31449953.3
64	2-Ethyltoluene	C9 H12	-1.36	120.09374	19.361	30295982.6
65	citraurin	C30	-3.05	432.30	22.0	26542433

		H40 O2		151	46	9.3
66	13(S)-HOTrE	C18 H30 O3	-2.07	294.21 889	18.6 58	25330666 1.7
67	Tapentadol	C14 H23 N O	-2.48	221.17 742	11.7 27	23608879 4.5
68	(8Z,11Z,14Z)-heptadecatrienoic acid	C17 H28 O2	-2.16	264.20 836	20.1 24	22990763 7.4
69	3,6-Anhydro-1-O-palmitoylhexitol	C22 H42 O6	-2.8	402.29 701	23.0 07	22128333 5.7
70	Linoleoyl ethanolamide	C20 H37 N O2	-4.16	323.28 108	17.4 16	21312311 6.3
71	Ferulic acid	C10 H10 O4	-4.12	194.05 711	10.4 86	20678170 9.5
72	5-Pentylresorcinol	C11 H16 O2	-2.77	180.11 453	13.8 36	20255445 6.5
73	9(Z),11(E),13(E)-Octadecatrienoic Acid methyl ester	C19 H32 O2	-3.78	292.23 913	20.9 54	19832593 1.6
74	(11E)-15-Oxo-11-icosenoic acid	C20 H36 O3	-3.31	324.26 537	21.9 24	19235388 1.8
75	1-(7,8-Dimethoxy-2,2-dimethyl-2H-chromen-6-yl)ethanone	C15 H18 O4	-2.79	262.11 978	17.0 26	19028834 5.4
76	8-Hydroxy-5,8a-dimethyl-3-methylene-3a,4,4a,7,8,8a,9,9a-octahydronaphtho[2,3-b]furan-2(3H)-one	C15 H20 O3	-3.04	248.14 049	18.3 9	18921651 0
77	1-Stearoyl-2-hydroxy-sn-glycero-3-PE	C23 H48 N O7 P	2.24	481.31 792	21.9 14	17290033 5.8
78	12-oxo Phytodienoic Acid	C18 H28 O3	-2.51	292.20 311	16.9 35	15142293 0.9

79	(2R)-3-Hydroxy-2-[(9Z,12E)-9,12-octadecadienoyloxy]propyl 2-(trimethylammonio)ethyl phosphate	C26 H50 N O7 P	-2.74	519.33 106	21.04	13616170 0.3
80	DL-carvone	C10 H14 O	-2.35	150.10 411	13.723	13504410 5.6
81	9(S)-HpOTrE	C18 H30 O4	-1.9	310.21 382	16.954	12713277 1.3
82	(15Z)-9,12,13-Trihydroxy-15-octadecenoic acid	C18 H34 O5	-2.25	330.23 988	16.711	12314870 9.5
83	3-oxopalmitic acid	C16 H30 O3	-2.37	270.21 885	20.857	11483045 0.8
84	6-Hydroxy-2,3,4-trimethoxybenzoic acid	C10 H12 O6	-2.35	228.06 285	1.482	11420563 4.8
85	2(N)-Methyl-norsalsolinol	C10 H13 N O2	-2.13	179.09 425	9.838	89627333 .47
86	4-methoxy-6-(prop-2-en-1-yl)-2H-1,3-benzodioxole	C11 H12 O3	-2.14	192.07 823	13.072	87383657 .19
87	Nicotinic acid	C6 H5 N O2	-2.03	123.03 178	21.422	82005166 .33
88	(9S,10S)-10-Hydroxy-9-(phosphonooxy)octadecanoic acid	C18 H37 O7 P	-3.27	396.22 639	20.03	75091596 .97
89	NP-015687	C19 H16 O4	-2.73	308.10 402	16.56	73785516 .74
90	Citral	C10 H16 O	-2.98	152.11 966	17.565	70786061 .68
91	Methylstyrylpyron	C14 H12 O4	-2.6	244.07 292	16.71	70357685 .76
92	2,3,4,9-Tetrahydro-1H-β-carboline-3-carboxylic acid	C12 H12 N2	-1.91	216.08 946	7.296	62222633 .61

		O2				
93	(2S)-2,3-Dihydroxypropyl (9Z)-9-hexadecenoate	C19 H36 O4	-3.42	328.26 024	19.8 19	61791481 .82
94	(+)-(S)-Carvone	C10 H14 O	-2.35	150.10 411	11.2 75	61539518 .31
95	1-Methyl-1,2,3,4-tetrahydro-Î²-carboline-3-carboxylic acid	C13 H14 N2 O2	-3.78	230.10 466	8.36 2	52753797 .35
96	cis-12-Octadecenoic acid methyl ester	C19 H36 O2	-3.7	296.27 043	20.5 45	51506683 .22
97	m-Cresol	C7 H8 O	0.05	108.05 752	17.8 69	51155504 .38
98	(2E,4E,7E)-2,4,7-Decatrienoic acid	C10 H14 O2	-2.37	166.09 899	10.7 09	51070344 .43
99	Ethyl oleate	C20 H38 O2	-4.8	310.28 569	22.2 41	50460851 .23
100	Apocynin	C9 H10 O3	-2.23	166.06 262	12.9 14	49615214 .04
101	(-)-isopiperitenone	C10 H14 O	-2.46	150.10 41	11.4 94	48182112 .4
102	NP-003672	C15 H14 O2	-2.89	226.09 873	14.5 55	46742344 .31
103	Ibuprofen	C13 H18 O2	-2.29	206.13 021	12.9 56	45378670 .95
104	4-(4-hydroxy-2-methoxy-3,5,6-trimethylbenzoyloxy)-2-methoxy-3,5,6-trimethylbenzoic acid	C22 H26 O7	-2.93	402.16 667	13.0 63	40793982 .98
105	Quercetin	C15 H10 O7	-2.64	302.04 185	13.5 47	39320299 .41
106	3,4-Dihydroxycinnamaldehyde	C9 H8 O3	-2.2	164.04 698	14.0 35	39215830 .21

107	N-[(2E)-3-(3,4-Dihydroxyphenyl)-2-propenoyl]tryptophan	C20 H18 N2 O5	-4.69	366.11 986	22.5 32	38726850 .42
108	Palmitic acid	C16 H32 O2	-2.97	256.23 947	21.6 12	38351961 .64
109	Corchorifatty acid F	C18 H32 O5	-2.37	328.22 42	15.4 49	36757398 .95
110	4-Ethylguaiacol	C9 H12 O2	-2.23	152.08 339	14.9 11	32907408 .94
111	trans-10-Heptadecenoic acid	C17 H32 O2	-2.66	268.23 952	17.8 61	32205740
112	3-dehydro-6-deoxoteasterone	C28 H48 O3	-4.18	432.35 854	21.7 31	30837864 .78
113	geranyl quinone	C16 H20 O2	-2.98	244.14 56	22.5 18	30206293 .08
114	NP-015559	C17 H14 O7	-3.03	330.07 295	15.1 86	27277921 .65
115	(10S)-Juvenile hormone III acid diol	C15 H26 O4	-2.79	270.18 236	14.9 34	24724986 .51
116	4-Coumaric acid	C9 H8 O3	-1.73	164.04 706	10.0 38	24696560 .89
117	10,16-Dihydroxyhexadecanoic acid	C16 H32 O4	-2.45	288.22 935	17.9 75	20103158 .23
118	COSMENE	C10 H14	-1.64	134.10 933	11.2 9	18817198 .48
119	3-Dimethylallyl-4-hydroxymandelic acid	C13 H16 O4	-3.89	236.10 394	13.8 79	18473927 .57
120	Levallorphan	C19 H25 N O	-1.7	283.19 313	11.4 99	18236209 .55
12	12-HSA	C18	-3.19	300.26	17.2	17713187

1		H36 O3		549	92	.6
12 2	(2Z)-2-(4-Hydroxybenzylidene)heptanoic acid	C14 H18 O3	-0.1	234.12 557	15.1 82	17317207 .37
12 3	Vitamin A	C20 H30 O	-2.89	286.22 884	20.7 65	16961516 .47
12 4	coenzyme Q2	C19 H26 O4	-3.9	318.18 187	18.4 9	15080090 .56
12 5	Octyl gallate	C15 H22 O5	-2.64	282.14 598	14.4 71	14564065 .2
12 6	4-Methoxycinnamaldehyde	C10 H10 O2	-2.5	162.06 767	10.7 71	14494446 .9
12 7	Guaiacol	C7 H8 O2	-0.66	124.05 235	9.20 7	14343201 .65
12 8	Methyl linoleate	C19 H34 O2	-3.97	294.25 471	20.6 86	14211480 .42
12 9	Eicosapentanoic acid	C20 H30 O2	-3.49	302.22 352	22.5 8	14208703 .13
13 0	Nandrolone	C18 H26 O2	-2.76	274.19 252	14.8 07	13243367 .08
13 1	(4E)-1-(4-Hydroxyphenyl)-4-decen-3-one	C16 H22 O2	-3	246.16 124	16.0 37	12478489 .91
13 2	2-Methoxyresorcinol	C7 H8 O3	-3.01	140.04 692	9.64 5	12411623 .58
13 3	7-Hydroxyflavan	C15 H14 O2	-2.89	226.09 873	11.6 53	11359843 .24
13 4	4,8,12-trimethyltrideca 1,3,7,11-tetraene	C16 H26	-2.81	218.20 284	20.8 16	9897947. 401
13 5	Eicosapentanoic acid	C20 H30 O2	-3.49	302.22 352	22.1 73	8676767. 851
13	3-Methyl-2-butenyl caffeate	C14	-1.47	248.10	13.2	8511089.

6		H16 O4		449	14	247
137	Thymoquinone	C10 H12 O2	-1.6	164.08 347	12.5 05	8440170. 481
138	Isorhamnetin	C16 H12 O7	-2.22	316.05 76	14.8 43	8058961. 505
139	2-Octenoic acid	C8 H14 O2	-1.16	142.09 921	10.8 07	7572010. 605
140	1-(3,4-Dihydroxyphenyl)acetone	C9 H10 O3	-2.23	166.06 262	10.7 6	6400114. 457

According to the table 3, more than a hundred active chemicals can be found when the active compounds in CXBCH preparations are examined using LC-HRMS. Linoleic acid, eremantin, anhydro-D-fructose, 1,5-Anhydro-6-deoxy-D-threo-hex-1-en-3-ulose, 1-linoyl glycerol, monoolein, Tur-merone, meglutol, and L-palmitin are the 10 most abundant active components in CXBCH. According to the results of the LC-HRMS analysis of the CXBCH preparations, the three primary components of CXE are turmerone (order 8), curcumin (order 13), and curcumene (order 15). Quercetin, the primary flavonoid, is ranked 105th, and thymo-quinone, the primary active ingredient in BCE, is ranked 137th.

3.2. Cytotoxic and Antioxidant Activity of CXBC Preparations

The antioxidant activity of CXBCH preparations was tested using the DPPH method while the cytotoxic activity test was carried out on T47D and Hela cells.

Radical scavenging activities CXBCH preparation

Figure 4 shows the ability of CXBCH, CXE, and BCE preparations as scavengers of free radicals from DPPH.

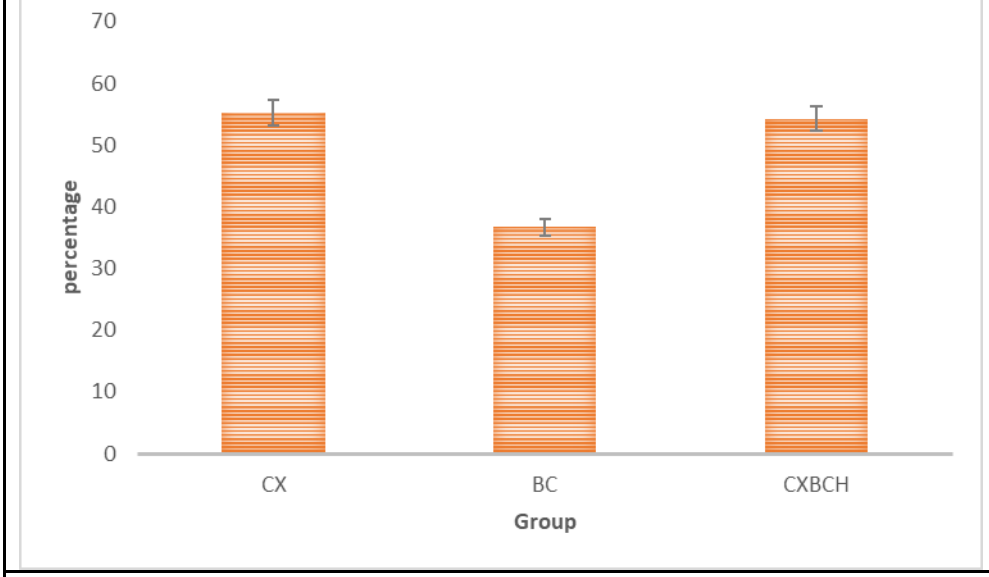
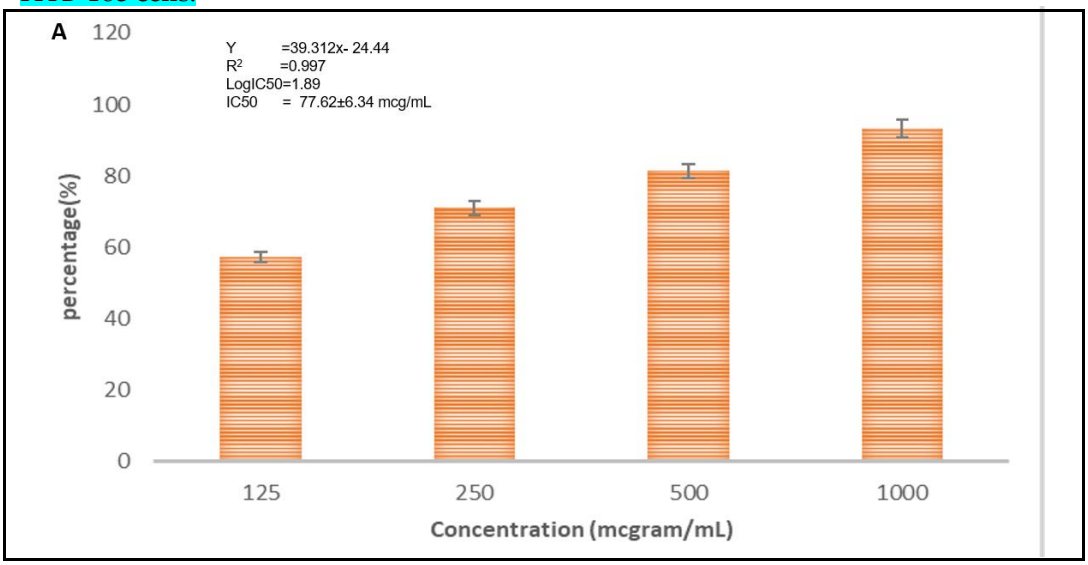


Figure 4. Antioxidant activity (IC50, mcg/ml) of CX extract, BC extract and CXBCH preparation using the DPPH method

Based on Figure 4, it can be seen that the ability (IC50) of the CXBCH preparation to bind free radicals was 54.26 mcg/mL.

Cytotoxicity activity of CXBCH preparation

Figure 5 shows that the CXBCH preparations inhibited the growth of T47D, Hela, HTB-183 cells.



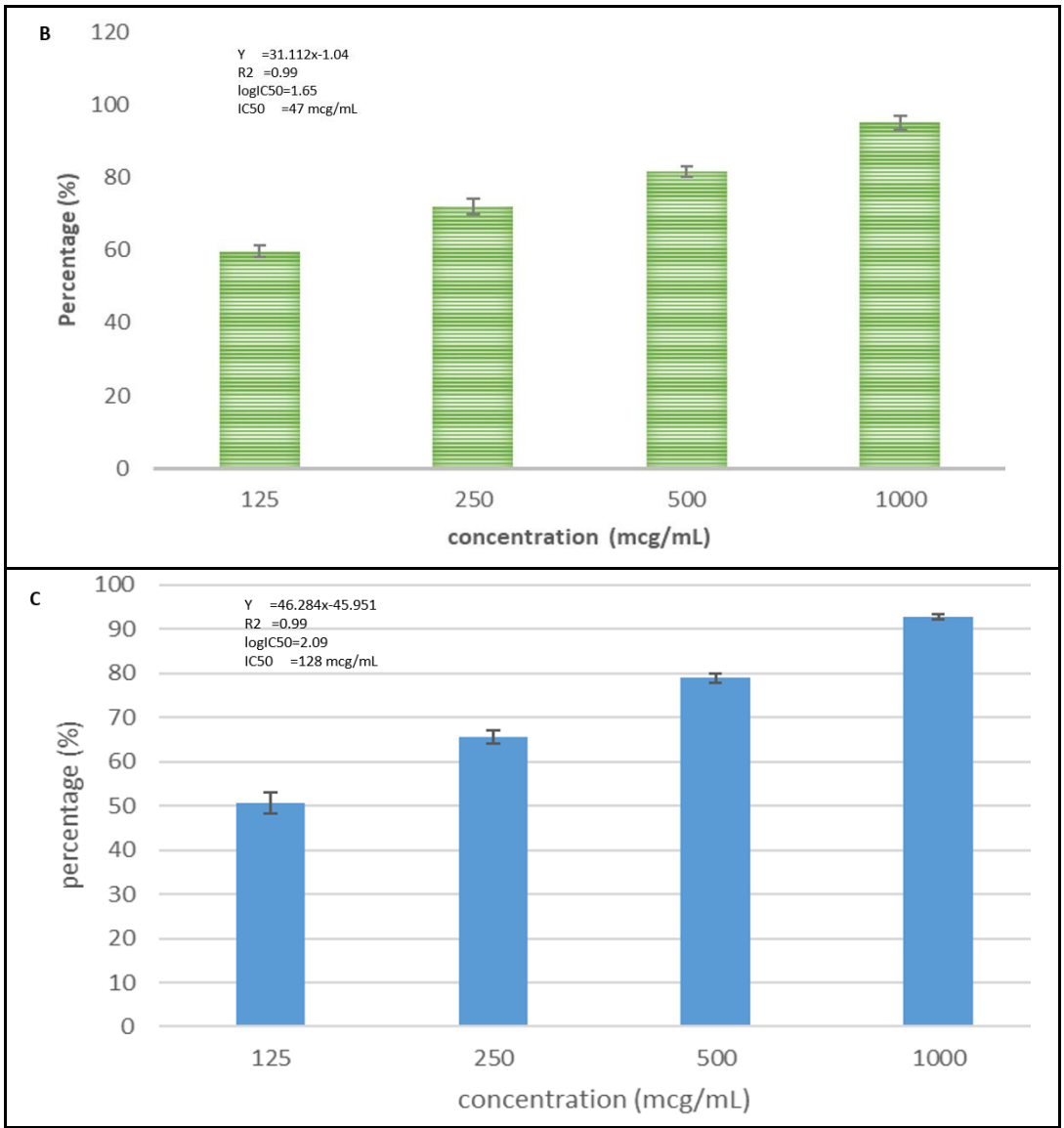


Fig.5. Inhibition activity of CXBCH preparation on T47D (A), Hela (B), and HTB-183 (C).

Based on the figure 5 we know that the CXBCH preparations inhibited the growth of T47D, Hela, HTB-183 cells with an IC50 of 77.62±4.66, 47.34±13.29, and 128±12.52 mcg/mL, respectively.

Furthermore, We have conducted an in vitro test to determine the mechanism of the chemopreventive action of CXBCH preparations by observing immunocytochemistry of p53 and caspase-3 expression on Hela cells. The results of testing the effect of CXBCH preparations on p53 expression in Hela cells are presented in the figure 6.

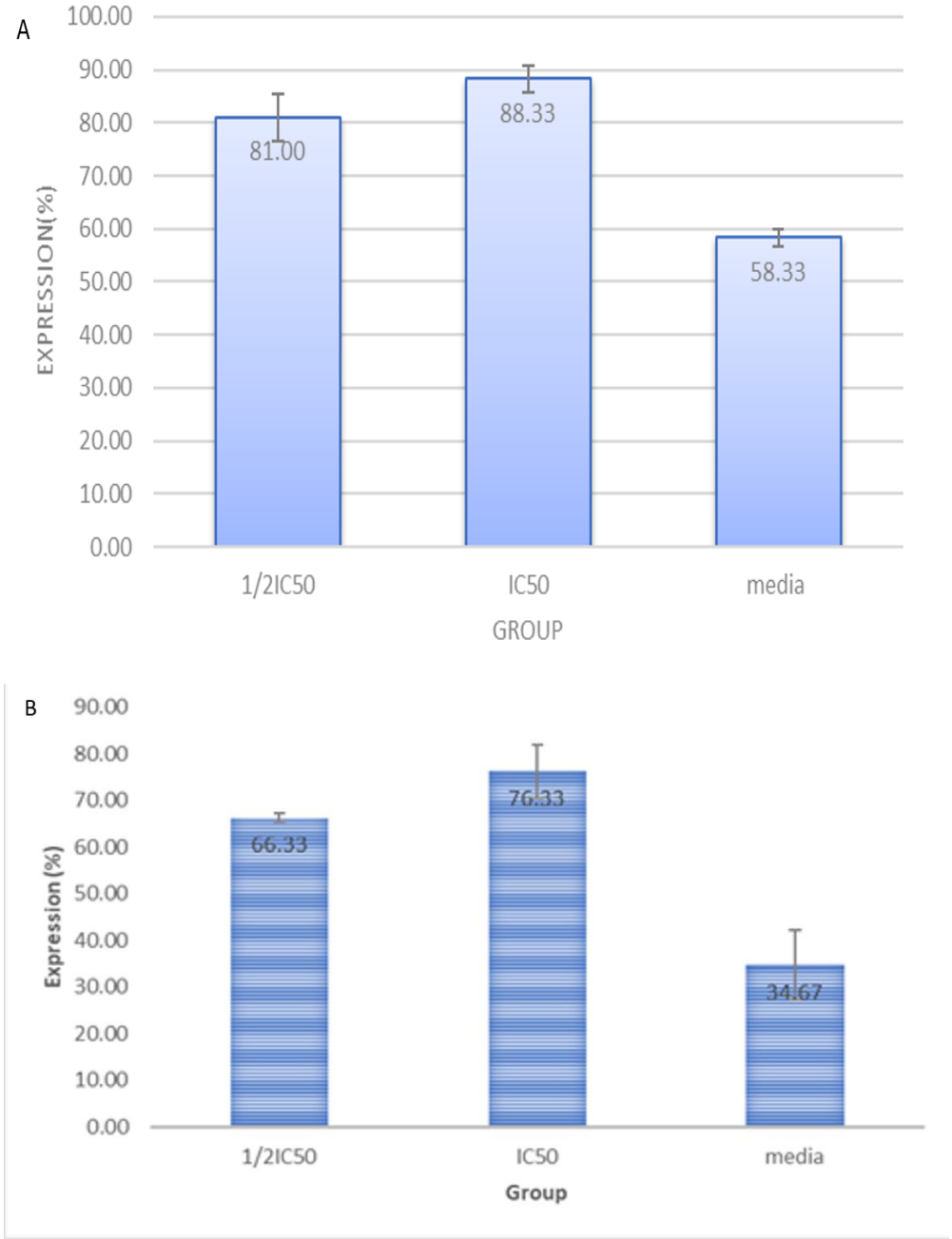


Fig. 6. Expression of p53 (A) and Caspase-3 (B) in Hela cells exposed to CXBCH preparations.

Caspase-3 is a crucial protein in apoptosis in addition to p53. Important mediators of programmed cell death are caspases. Among these, caspase-3 is a death protease that is regularly activated and catalyzes the precise cleavage of numerous essential cellular proteins. The results of the experiment demonstrated that the CXBCH preparations boosted the expression of caspase-3 and p53.

3.3. CXBCH Chemopreventive Activity in SD Rats

3.3.1. Clinical Conditions of Test Animals

Based on measurement in the first week, the average body weight of the SD rats was virtually similar between groups ($p>0.05$). In general, the average body weight from the first week to the 26th week increased but from the 26th to the 30th week it decreased. The

average body weight in the DMBA and tamoxifen groups from the 26th to the 30th week was the lowest, but not statistically significant ($p>0.05$).

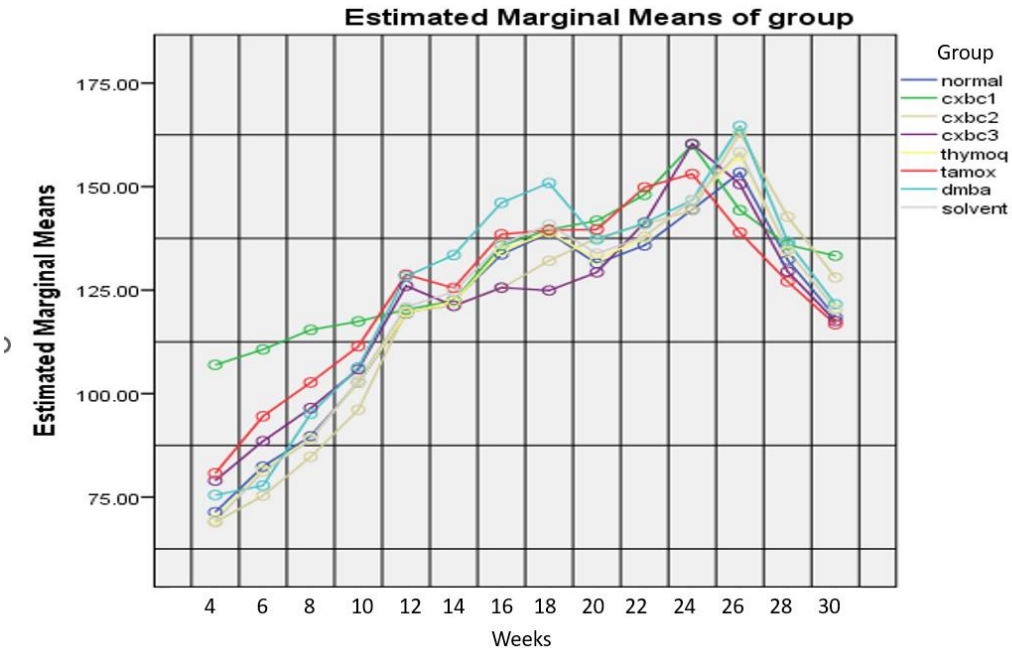


Fig 7. Development of DMBA-induced SD rat body weight by administering CXBCH preparations.

Table 4 presents the survival ability of DMBA-induced female SD rats with CXBCH treatment.

Table 4. Results of observations of the survival ability of each group of SD rats receiving CXBCH two weeks before and five weeks during DMBA induction.

Test Groups	**n**	**Dead beginning**	**Livability of test animals (%)week**			**Percentage of total deaths (%)**
			16	20	30	
Normal	10	1	90.00	90.00	90.00	10.00
CXBCH 1	10	1	90.00	90.00	90.00	10.00
CXBCH 2	10	2	80.00	80.00	80.00	20.00
CXBCH 3	10	1	90.00	90.00	80.00	20.00
Thymoquinone	10	1	90.00	90.00	90.00	10.00
Tamoxifen	10	2	80.0	80.00	70.00	30.00
DMBA	10	3	70.00	40.00	30.00	100.00
Solvent	10	1	90.00	90.00	90.00	10.00

Table 4 illustrates that the DMBA group had the lowest survival rate (0%) followed by the tamoxifen group with three deaths (70%), and the CXBCH2 group with two deaths (80%), while the CXBCH3 group had the highest ($p<0.05$ The CXBCH1, the normal, the solvent control, and the thymoquinone groups all had the highest livability rate (90%), with only one death in each group. These results indicate that DMBA induction increased the risk of death and the CXBCH administration increased the survival rate.

The examination results of peripheral blood, kidney and liver function of SD rats are presented in Table 5 and 6. DMBA induction at 10x20mg/kg BW, 2x/week for five weeks in female SD rats, reduced hemoglobin (Hb), mean corpuscular volume (MCV), mean corpuscular (MCH), and blood cells, but increased the levels of SGPT/SGOT and urea/creatinine. This study proved that DMBA induction suppressed bone marrow hematopoiesis or hematotoxicity. The number of leukocytes, erythrocytes, platelets, Hb, MCV, and MCH in the DMBA group was lower than that in the normal group ($p<0.05$). The administration of CXBCH, thymoquinone, and tamoxifen for two weeks before and five weeks during DMBA induction increased cell count, Hb, MCV, and MCH, as the average leukocyte, erythrocyte, platelet, Hb, MCV, and MCH counts of the treatment groups were higher than that of the DMBA group ($p<0.05$).

Table 5. Routine blood test results of DMBA-induced SD rats and CXBCH treatment two weeks before and five weeks during induction. Blood sampling was carried out at the 30th week of the experiment.

Group	Leukocyte count (x103/µL) (mean±sd)	Erythrocyte count (x106/µL) (mean±sd)	Platelet count (x103/µL) (mean±sd)	Hb level (mean±sd)	MCV (mean±sd)	MCH (mean±sd)
Normal(10)	6.33±1.37*	8.32±0.40*	981.33±95.37*	14.67±0.52*	58.33±1.37	20.00±0.89*
CXBCH 1 (10)	9.86±0.90a*	7.87±0.27*	668.71±50.91*	14.74±0.24*	5686±0.89	18.71±0.49*
CXBCH 2 (10)	6.29±0.49*	7.35±0.28*	724.71±181.25*	14.44±0.80*	59.29±0.49	19.29±0.49*
CXBCH 3 (10)	7.00±0.93*	7.65±0.14*	802.00±106.23*	14.59±0.85*	59.38±0.52	19.38±0.52*
Thymoquinone (10)	6.86±0.90*	6.53±0.94a*	734.29±151.14*	13.34±0.74*	57.34±2.30	21.17±1.21*
Tamoxifen(10)	7.00±2.68*	6.59±0.21a*	889.17±387.24*	13.83±2.99*	57.33±1.63	19.67±0.52*
DMBA(10)	2.80±1.10a	4.18±0.94a	255.00±70.31a	6.60±2.07a	54.40±1.52a	16.33±0.52a
Solvent (10)	6.67±1.03*	9.14±0.42*	908.00±120.10*	15.67±0.52*	56.67±1.37	19.33±0.52*
Reference**	7.67 ± 1.62	8.20±0.55	836.00±132.00	15.4±0.90	53.6±1.70	19.00±0.60

Note:a=<0.05 for the normal group; *=p<0.05 for the DMBA group; **= (Giknis, 2008).

The results of renal and hepatic physiology examination are presented in Table 6, Showing that DMBA induction is nephrotoxic and hepatotoxic. However, the serum urea and creatinine levels in this study differ from the referenced study (Giknis, 2008). DMBA induction increases serum urea and creatinine levels, as shown by the significantly higher levels of urea and creatinine levels in the DMBA group compared to the normal group ($p<0.05$). Furthermore, the average levels of SGPT and SGOT in the DMBA group were also 3x and 7x higher respectively than those in the normal group ($p<0.05$).

Table 6. Results of examination of blood urea and creatinine levels of DMBA-induced SD rats after receiving CXBCH treatment two weeks before and five weeks during DMBA induction. Blood sampling was carried out at the 30th week of the experiment.

Test Group	Serum urea level (mean±sd) (mg/dl)	Serum creatinine level (mean±sd) (mg/dl)	The average level of SGPT(mean±sd) (U/L)	Average serum SGOT level mean±sd)(U/L)
Normal(10)	26.33±1.37*	0.40±0.00*	44.00±0.89*	130.33±1.37*
CXBCH 1 (10)	27.14±4.81*	0.26±0.05*	52.00±4.86*b	107.19±21.78*
bCXBCH 2 (10)	30.29±2.06*	0.30±0.00*	58.00±11.65*b	88.00±4.89*b
CXBCH 3 (10)	33.63±4.17*	0.30±0.00*	55.38±9.04*b	83.50±3.70*b
Thymoquinone (10)	31.11±7.26*	0.34±0.05*	44.31±12.81*b	124.14±5.46*b
Tamoxifen(10)	37.00±6.39	0.35±0.08*	95.33±74.29a*	206.17±2.43a*
DMBA(10)	40.80±0.84[a]	0.54±0.05a[a]	156.80±50.58[a]	830.40±92.66[a,b]
Solvent (10)	35.00±4.73	0.33±0.05*	60.33±7.23*	92.00±8.80*
Reference**	17.50±3.90	0.40±0.10	30±15	101±36

Note: a=p<0.05 for the normal group; *=p<0.05 for the DMBA group; **= (Giknis, 2008).

Administration of CXBCH for two weeks before and five weeks during DMBA induction in SD rats was shown to be nephroprotective and hepatoprotective. The average urea and creatinine levels in the CXBCH groups were lower than those in the DMBA group ($p<0.05$). The mean blood urea and creatinine levels in the thymoquinone group did not differ from those in the CXBCH groups ($p>0.05$) but those in the tamoxifen group were higher ($p<0.05$). The average levels of SGPT and SGOT in the CXBCH groups were significantly lower than those in the DMBA group ($p<0.05$). It appears that administration of CXBCH has decreased SGPT and SGOT levels in DMBA-induced SD rats, 66% and 90% respectively.

3.3.2. Nodule formation

The examination results of the percentage of nodule formation, the number of nodules per group, and the nodule weight are presented in Table 7 and Figure 8.

Table 7. The examination results of the number of nodules in DMBA-induced female SD rats receiving CXBCH treatment two weeks before and five weeks during induction.

Test Group (n)	Incidence of tumor formation (%)	Number of nodules formed	Tumor multiplicity (nodule/rat)	Total weight of nodules (grams)
Normal (10)	0	0	0.0±0.0	0
CXBCH 1 (10)	50%	8	0.50±0.50	2.51
CXBCH 2 (10)	50%	8	0.80±0.92	3.25
CXBCH 3 (10)	50%	6	0.73±0.79	4.17
Thymoquinone (10)	30%	3	0.30±0.48	1.20
Tamoxifen (10)	30%	5	0.46±0.93	1.40
DMBA(10)	100%	14	1.40±1.1	10.53
Solvent (10)	0	0	.0.0±0.0	0

The DMBA group had the highest percentage of nodule formation, with 100%. All the SD rats in the DMBA group were successfully induced with DMBA and all formed tumor nodules (100%). The administration of CXBCH to DMBA-induced female SD rats reduced the percentage of nodule formation per group, as shown by the lower percentage of nodule formation in the CXBCH treatment groups. Furthermore, the thymoquinone and tamoxifen groups had the lowest nodule formation percentages, with 28% and 30% respectively. Therefore, the CXBCH administration for two weeks before and five weeks during DMBA induction inhibited the formation of tumor nodules in SD rats.

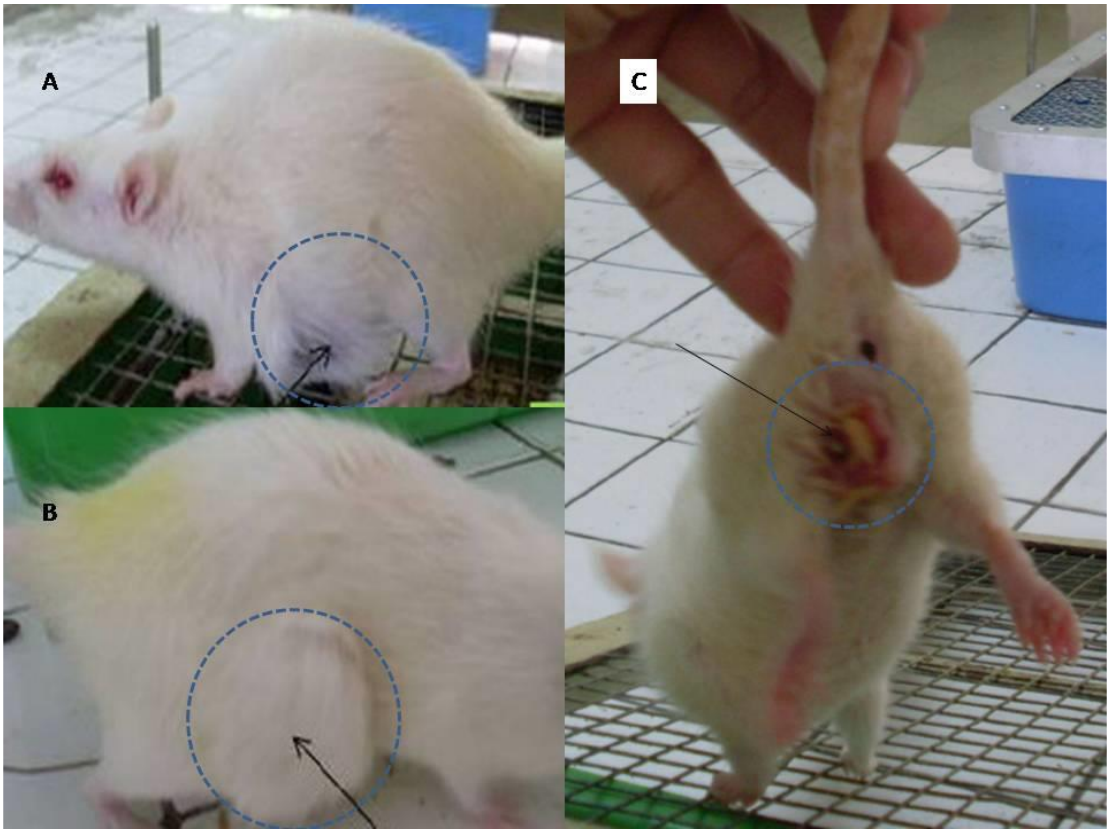


Figure 8. Nodules (arrows) were formed in the mammary gland in the 20th week of observation of SD rats after receiving CXBCH 2 weeks before and five weeks after DMBA induction. Nodules formed in all parts of the mammary gland (fore legs (A), near the hind legs (B), and some nodules break up (C). Note: Arrows indicate nodules

Based on the number of nodules formed per group, the DMBA group had the highest number, with 14 nodules. The number of nodules in the CXBCH1 (8 nodules), CXBCH2 (8 nodules), CXBCH3 (6 nodules), thymoquinone (3 nodules), and tamoxifen (5 nodules) groups was lower than that in the DMBA group. Among the groups that received CXBCH, CXBCH3 had the least number, with six nodules. The results of this study indicate that DMBA can induce the formation of tumor nodules in the mammaries of SD rats, similar to what has been reported by previous researchers.

Based on the time of tumor nodule formation, the earliest nodule formation occurred in the DMBA group, namely at the 10th week, followed by the CXBXH1 group at the 14th week, the CXBCH3 group (17th week), CXBCH2 (18th weeks) and tamoxifen (18th week). The thymoquinone group had the most recent formation of tumor nodules, namely after the 20th week. Among the treatment groups that received CXBCH, nodule formation was formed the fastest in the CXBCH1, followed by the CXBCH3, and CXBCH2.

3.2.3. Histopathological Examination of Tumor Tissue in SD Rats Induced with DMBA

Figure 9 and Table 8 present the histopathological observations of carcinogenesis in mammary tissue tumor nodules. None of the SD rats were diagnosed with mammary carcinoma (adenocarcinoma) in the solvent and normal groups as microscopically, the mammary gland cells of SD rats in these groups showed normal mammary tissue histology. There was no change in the histology of mammary tissue characterized by hyperplasia, metaplasia, and neoplasms. Meanwhile, the mammary tissue of SD rats in the

DMBA group, which experienced carcinogenesis and formed tumor tissue, showed hyperplasia of the connective tissue. The connective tissue was denser due to pressure from the enlarged tumor cells. Neoplasm cells from the ductal epithelium (adenocarcinoma) and acini were seen as well as the presence of inflammatory cells and collections of necrotic cells (Figure 9).

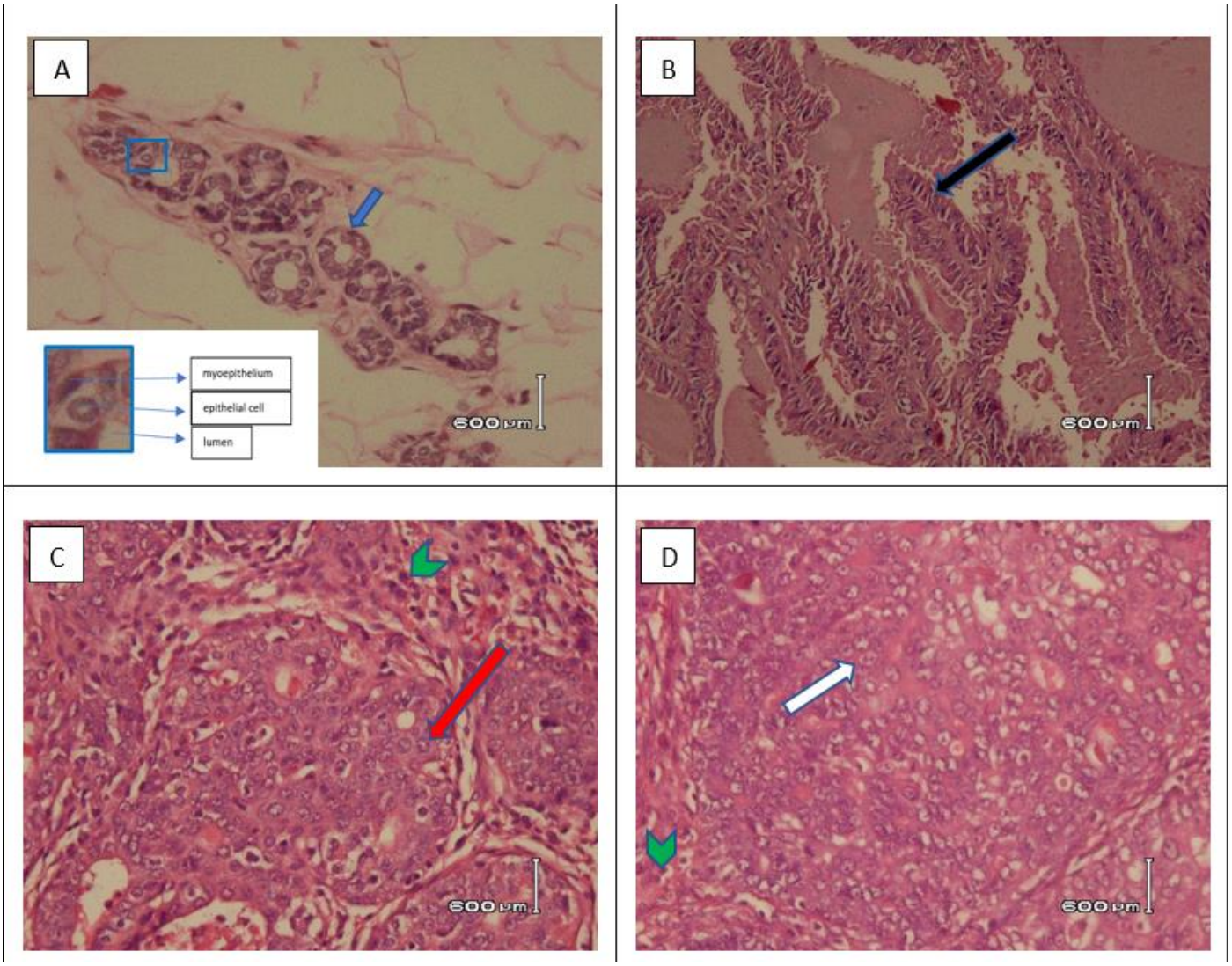


Figure 9. Microscopic view of mammary tissue of SD rats induced by DMBA by giving CXBC for two weeks and five weeks during induction (HE staining).Notes: (A) Normal tissue (400x). Mammary gland ducts are composed of non-atypia epithelial cells and myoepithelium (insert), (B) Tissue with papillary carcinoma (100x). Delicate papillary fronds (black arrow) and expansile papillary tumor, with low or intermediate grade nuclei, cuboidal to columnar epithelial cells that lacks myoepithelial cells along the papillae and at the periphery or shows focal peripheral myoepithelial staining. (C) Tissue with Ductal carcinoma in situ (400x). An intraductal epithelial proliferation with intermediate grade nuclear atypia (red arrow), tubular and cribriform growth pattern with sufficient lymphocyte reaction (green arrow head) (D) Tissue with invasive ductal carcinoma of no special type (NST) (400x). The preparation shows solid and tubular epithelial tumor tissue, infiltrative to the surrounding connective tissue. Tumor cells are atypia, polymorphic, large size. Cytoplasm a little until enough. The nuclei (white arrow) are large, pleomorphic, round, oval, polygonal, irregular chromatin, partly vesicular with nucleolus are clearly visible. Mitosis is slight. Lymphocyte reaction is slight (green arrow head).

The study found that seven SD rats in the DMBA group ultimately all had nodules (100%), which were 100% histopathologically diagnosed as adenocarcinoma. Administration of CXBCH for two weeks before and five weeks during DMBA induction could inhibit the carcinogenesis process (Figure 9). Histopathological examination of mammary

tissues in the treatment groups with various CXBCH doses showed tumors with adenocarcinoma, tumors without adenocarcinoma, and those that showed hyperproliferation and normal.

Table 8. Histopathological examination of mammary tissue with or without mammary tissue tumor nodules in SD rats induced with DMBA and receiving CXBCH treatment two weeks before and five weeks during induction. Tissue collection was carried out at week 30.

Test Groups	Type of histopathological picture (%)			% Inhibition of ACM
	TAP	PP	ACM (invasive)	
Normal (10)	100.00	0	0	-
CXBCH 1 (10)	50.00	30.00	20.00	80
CXBCH 2 (10)	60.00	10.00	30.00	70
CXBCH 3 (10)	55.00	40.00	10.00	90
Thymoquinone (10)	70.00	30.00	0	100
Tamoxifen (10)	72.70	0	27.30	73
DMBA(10)	0	0	100.00	0
Solvent (10)	100	0	0	0

Notes: TAP=no change (normal); PP=proliferation; ACM=adenocarcinoma.

Histopathological examination of the mammary tissues in the CXBCH treatment groups revealed that there were various forms of epithelial proliferation, both epithelial from the acini and ductal epithelium. However, some of the hyperplastic features seen in the thymoquinone, tamoxifen, CXBCH1, CXBCH2, and CXBCH3 groups could not be classified as mammary adenocarcinoma. Histopathological picture of the hyperplastic epithelium was found in the treatment groups that received CXBCH1 (30%), CXBCH3 group (40%), and CXBCH2 (10%). In the thymoquinone group, the hyperplastic picture was 30%. Hyperplasia was not found in the tamoxifen and DMBA groups. In the mammary gland tissues, ductal adenocarcinoma in situ and invasive was found. As observed in the CXBCH and tamoxifen groups, anaplastic cells were still restricted to the lobules and the ductal basement membrane remained intact in adenocarcinoma in situ. The percentage of adenocarcinoma formation among SD rats that received successive CXBCH treatment was the lowest in the CXBCH3 group at 10%, the CXBCH1 group at 20%, and the CXBCH3 group as the highest at 30%. The DMBA group exhibited the highest percentage of invasive cancer on histopathology (100%) compared to the thymoquinone group (0%). This study demonstrated that CXBCH injection slowed the progression of carcinogenesis in DMBA-induced SD rats, indicating that CXBCH could potentially serve as a chemopreventive drug.

3.4. CXBCH by Increasing GST Activity and Decreasing Serum NO Levels

The results of an examination of serum NO levels of SD rats at the 30th week of treatment are presented in Table 9. The results indicate that CXBCH1, CXBCH2, and CXBCH3 administration for seven weeks to female DMBA-induced SD rats decreased serum NO levels.

Table 9. Serum NO levels in SD rats treated with CXBCH two weeks prior to and five weeks during DMBA induction at week 30.

Test Group	Average serum NO level (µM)(mean±sd)
Normal (10)	0.20±0.07*
CXBCH 1 (10)	0.21±0.05*
CXBCH 2 (10)	0.24±0.05*
CXBCH 3 (10)	0.18±0.12*
Thymoquinone (10)	0.29±0.09*
Tamoxifen (10)	0.23±0.01*
DMBA(10)	0.38±0.09a

Solvent (10)	0.18±0.02*

Note.: *: Significant ($p<0.05$) for the DMBA group;.

DMBA induction at 2x20 and 10x20 mg/kg BW in SD rats increased serum NO levels. The results of this study proved that the serum NO levels in the DMBA group at the fourth week of measurement were higher than those in the normal and solvent control groups ($p<0.05$). Likewise, at the 30th week of measurement, the serum NO level of the DMBA group was higher than that of the normal and solvent control groups ($p<0.05$). Administration of CXBCH, thymoquinone, and tamoxifen was shown to reduce serum NO levels of DMBA-induced SD rats. At the 30th weeks of measurement, the serum NO level of the treatment group receiving CXBCH, thymoquinone, and tamoxifen was lower than that of the DMBA group ($p<0.05$).

CXBCH preparations increased the activity of GST enzymes in the liver and spleen,

as presented in Table 10. In general, this study proved that the liver GST enzyme activity in the DMBA group was lower than the regular liver GST activity ($p<0.05$). In other words, DMBA induction decreases hepatic GST enzyme activity.

Table 10. GST activity of the liver and spleen of SD rats that received CXBCH two weeks before and five weeks during DMBA induction at week 30.

Group	GST activity in liver and spleen tissue (mean±sd) (ug/min/ml)	
	Spleen	**liver**
Normal (10)	8.70±0.89*	82.91±7.93*
CXBCH 1 (10)	17.39±2.17a*	106.98±5.45a*
CXBCH 2 (10)	18.87±1.30a*	112.21±8.87a*
CXBCH 3 (10)	20.44±0.98a*	113.83±10.08a*
Thymoquinone (10)	18.74±2.38a*	91.14±7.18a*
Tamoxifen (10)	17.62±2.61a*	83.29±11.14*
DMBA(10)	6.86±0.91a	65.54±3.31a
Solvent (10)	8.41±0.76*	83.50±7.31*

Note.: a=<0.05 for the normal group; *: Significant ($p<0.05$) to the DMBA group

CXBCH administration increased the activity of the liver GST enzyme in SD rats, as the average liver GST enzyme activity of the CXBCH groups was higher than that of the DMBA group ($p<0.05$). It also increased the activity of the liver GST enzyme in SD rats that were not induced with DMBA. CXBCH administration also increased the GST enzyme activity in SD rats induced with DMBA at 2x20 or 10x20 mg/kg BW. Observation of liver GST enzyme activity at the 30th week of treatment proved that DMBA induction decreased liver GST enzyme activity and CXBCH administration increased it. The GST enzyme activity of the DMBA group was lower than that of the standard group ($p<0.05$) while the treatment groups that received CXBCH, thymoquinone, and tamoxifen showed higher GST activity than the DMBA group ($p<0.05$).

3.5. CXBC Preparations Increase the Number of CD4, CD8 and CD4CD25.

Table 11 presents the results of the flow cytometry examination. It can be seen that DMBA induction at 10x20 mg/kg BW decreased absolute CD4 and CD4CD25 counts as the counts in the DMBA group was lower than those in the standard group ($p<0.05$), with only a third. The absolute CD4CD25 count in the DMBA group was also lower ($p<0.05$), but the ratio of CD4CD25 to CD4 in the DMBA group was higher than the that in the standard group ($p<0.05$).

Table 11. Examination results of the absolute number of CD4 lymphocytes in the peripheral blood of SD rats induced with DMBA at 2x20 mg/kg/week for five weeks after receiving seven weeks of CXBCH treatment. Blood sampling was carried out at the 30th week of the experiment.

Test group	**Absolute amount**	**Absolute CD4CD25**	**Percentage of CD4CD25**

	CD4 (mean±SD)	count(mean±SD)	to CD4 (mean±SD
Normal(10)	1575.67±131.70*	70.50±11.76	4.41±0.01*
CXBC 1 (10)	1619.57±519.86*	80.86±17.78*	4.19±0.01*
CXBC 2 (10)	1868.57±382.55*	113.32±20.58a*	6.14±0.01*
CXBC 3 (10)	1668.75±398.01*	92.50±20.53a*	5.66±0.01*
Thymoquinone (10)	1799.83±429.90*	97.50±21.69a*	5.62±0.02*
Tamoxifen(10)	1940.00±203.76*	84.50±13.46a*	4.34±0.00*
DMBA(10)	484.17±33.98a	45.17±9.07a	11.50±0.02a
Solvent (10)	1490.33±508.98*	109.33±64.06*	7.35±0.04*

Note. a=p<0.05 for the normal group: *=p<0,05 to the DMBA group.

CXBCH administration increased the absolute number of CD4 and CD4CD25 but decreased the percentage of CD4CD25/CD4. The CD4 and CD4CD25 counts in the CXBCH group were higher than those in the DMBA group (p<0.05), but the CD4CD25/CD4 percentage in the CXBCH group was lower (p<0.05). Among the treatment groups, the CXBCH2 group had the highest absolute numbers of CD4 and CD4CD25, followed by the CXBCH3 group and CXBCH1. The mean absolute CD4 count of the CXBCH2 group was almost the same as that of the thymoquinone group (P>0.05). CXBCH administration for two weeks before and five weeks during DMBA induction reduced DMBA's immunotoxic effect on CD4 and CD4CD25 counts. The CXBCH's ability to inhibit the immunotoxic effects of DMBA was equivalent to that of thymoquinone at a dose of 50 mg/kg (p>0.05).

CXBCH increases absolute CD8 and CD8CD25 counts

Table 12 presents the absolute number of CD8 and CD8CD25 and the percentage of CD8CD25/CD8. It shows that DMBA induction reduced the absolute number of CD8 and CD8CD25, namely to ¼ of standard CD8 number and 3/5 of standard CD4CD25 number, but increased the percentage of CD8CD25 to CD8 (p<0.05), although the percentage was still higher than the DMBA group (p<0.05).

Table 12. The absolute number of peripheral blood CD8 lymphocytes of SD rats induced with DMBA at 2x20 mg/kgBW/week for five weeks after receiving CXBCH treatment for seven weeks. Blood sampling was carried out at the 30th week of the experiment.

Test Group	Absolute CD8 count (mean±SD)	The absolute number of CD8CD25(mean±SD)	Percentage of CD8CD25 to CD8 (mean±SD)
Normal(10)	580.00±66.63*	52.50±9.39*	9.00±1.30*
CXBCH 1 (10)	840.43±48.64*	77.00±2.15*	10.70±3.67*
CXBCH 2 (10)	860.00±33.95*	85.28±2.6*	10.84±3.00*
CXBCH 3 (10)	512.50±47.42*	53.00±3.86*	13.18±3.72*
Thymoquinone (10)	813.33±18.17*	67.16±16.77*	8.70±3.09*
Tamoxifen(10)	915.00±17.13*	68.16±20.62*	7.52±2.32*
DMBA(10)	137.00±18.48a	32.66±6.43a	23.75±2.5a
Solvent (10)	668.33±39.56*	59.33±16.94*	10.74±5.6*

Note:a=p<0.05 for the normal group; *=p<0.05 to the DMBA group

CXBCH administration to DMBA-induced SD rats increased the CD8 and CD8CD25 counts but decreased the percentage of CD8CD25 to CD8. Total CD8 and CD8CD25 were found to be higher in the CXBCH group than those in the DMBA group (p<0.05), but the ratio of CD8CD25 to CD8 was lower. (p<0.05).

4. Discussion

This study aimed to determine the effectiveness of CXBCH preparations as chemopreventive, antioxidant, and immunomodulator in DMBA-induced SD rats. The novelty in this publication is the test material in the form of herbal honey preparations (CXBCH) containing Curcuma xanthorriza extract(CXE) and black cumin extract (BCE).

CXBCH Preparation Ingredients and Activities

Thymoquinone and curcumin are the main active substances in the CXBCH preparations. According to the results of the analysis of the CXBCH's active ingredients, in addition to thymoquinone and curcumin, CXBCH preparations also contain fructose, eremantin, meglutol, monoolein, tur-meron, and palmitin. Thymoquinone is BC's main active substance while curcumin is CX's active substance. The composition of the active substances in BC and CX extracts is determined by the extraction method, the type of solvent compound, and the region of origin[62]. Making CXBCH preparations by utilizing honey as a solvent and flavoring medium can overcome the weaknesses of BC oil preparations, which often cause burping, an unattractive taste, and a pungent aroma[63][64]. The high levels of thymoquinone and curcumin in CXBCH preparations, accompanied with a pleasing sweet taste, indicates that the CXBC herbal honey preparations are in line with the expectations of both researchers and consumers[45][65].

Thymoquinone and curcumin have been shown to have various biological activities[66][34]. Nigelon is a polymer form of thymoquinone that inhibits the activity of cyclooxygenase and lipoxygenase enzymes in arachidonic metabolism; hence, it is believed that it can be employed as an analgesic, anti-allergic, anti-inflammatory, and anticancer agent[67]. Thymoquinone has also been demonstrated to be hepatoprotective[68], antioxidative[69], neuroprotective due to ischemia[70], antihyperlipidemic[71], nephroprotective[72], immunomodulatory by inhibiting NFkB[73], anti-autoimmune disease agent[74], and anti-cancer[75] .

CXBCH chemopreventive activity

The research data showed that DMBA induction at 10x20 mg/kgBW resulted in nodule formation and carcinogenesis. Administration of CXBCH preparations, thymoquinone, and tamoxifen, has been shown to inhibit such formation as the results showed that the formation and the number of nodules in the CXBCH, thymoquinone, and tamoxifen groups were lower than those in the DMBA group. This study is in line with the activity of thymoquinone and curcumin as antioxidants and anti-inflammatories, thereby reducing the formation of the active DMBA metabolite (DMBA-DE)[76][77].

The CXBCH content is thought to inhibit the meeting of AhR with DMBA (ligand), as there is no activation of the signal transduction pathway of AhR, and no active metabolite of DMBA-DE is formed [55][78][35]. Thymoquinone, dithymoquinone, dihydro-thymoquinone, unsaturated fatty acids, and sitosterol are compounds, with a molecular structure similar to AhR ligands [56], can act as AhR ligands as partial antagonists/agonists and are competitive against DMBA [57]. Active CXBCH preparations, such as polyphenols, flavonoids, curcumin, and thymoquinone, can competitively block the junction of DMBA with AhR, preventing the formation of the DMBA-AhR complex and preventing AhR receptor activation. There are insufficient cytochrome CYP1A1/CYP1B1 enzymes for the metabolism of DMBA to DMBA-DE because the ligand-receptor complex (DMBA-AhR) does not form, preventing AhR from translocating as a transcription factor and preventing the transcription of the CYP1A1/CYP1B1 gene[58][79]. Thymoquinone, flavone, and epigallocatechin (EPGK) activity of several scavenger substances has been compared[80]. Thymoquinone's ability to scavenge or neutralize free radicals in skin is comparable to that of the polyphenol molecule found in tea59][81].

CXBC antioxidant activity through increased GST expression and decreases NO levels

The results showed that CXBCH preparations decreased NO levels and increased GST levels. DMBA induction has been shown to increase plasma NO levels as the level was higher in the DMBA group than that in the standard and solvent groups ($p<0.05$). Genotoxic stress is the cells' response to the presence of DNA-damaging agents both from extracellular and intracellular sources, such as NO[82]. It can cause genetic changes and cell damage [60][83]. Mammalian cells have biochemical components as a defense system to maintain cell integrity from stressors both inside and outside the cells, includ-

ing the antioxidant cytoprotective enzyme GST (Phase II) [17][84]. CXBCH preparations, such as thymoquinone and tamoxifen, could reduce NO levels and increase GST activity in DMBA-induced SD rats. The antioxidant mechanism of CXBCH can be explained by the results of this study which showed that DMBA induction decreased GST enzyme activity, while CXBCH administration before and during DMBA induction increased GST enzyme activity. The biochemical content of CXBCH appears to work directly in increasing the production of the GST enzyme[85]. These results align with those of previous studies, which have proven that the bioactive content of BC shows activity as a phase II enzyme promoter in both in vitro and in vivo tests [62][86][87]. The data from this study and the evidence from previous studies show that the antioxidant activity of CXBCH and thymoquinone is a promoter of GST gene activation, so the production of GST enzymes increases[84]. As a scavenger, thymoquinone and other active CXBCH substances can bind directly to the reactive radical, DMBA-DE, formed from phase I metabolism so that it is not reactive [36]. The rapid reaction between thymoquinone and GSH produces a reduced compound glutathione dihydro-thymoquinone (GDHTQ) whereas the slow reaction of thymoquinone with NADH and NADPH produces a reduced compound dihydro-thymoquinone (DHTQ)[88]. The antioxidant activity of DHTQ and GDHTQ as scavengers against active organic radicals (DPPH) is the same, while the ability of thymoquinone is lower[36][89].

CXBCH as Immunomodulator

Research has shown that DMBA induction causes oxidative stress and is immunosuppressive, as evidenced by a decrease in the number of leukocytes and lymphocytes and a decrease in the activity of GST enzymes in the liver and spleen. Administration of CXBCH preparations has been shown to increase the cellular components of blood and the number of lymphocytes. The CXBCH groups had higher CD4, CD8, and CD4CD25 cells than the DMBA group ($p<0.05$). The results of this study are in accordance with those of previous studies, which show that DMBA and other xenobiotic PAHs result in the formation of reactive radicals that are immunotoxic[23] [90], while BC and CX increase immune responses or are immunostimulant and antioxidative[91][92][93]. Curcumin has been shown to influence CD4Th differentiation in vivo[94]. Like curcumin, thymoquinone has increased the number of CD4Th lymphocytes in vivo [68][95]. Thymoquinone has been shown to increase macrophage activity by activating Toll-like receptors (TLRs) [69][96]. Thymoquinone and other active substances from Nigella sativa have been shown to increase lymphocyte proliferative activity, macrophage activity, and IFN-γ production in vivo [70][97][95].

Although it has been anticipated, this research still has some weaknesses. Due to technical limitations and problems, the researchers did not measure DMBA-adduct as a biomarker of genotoxic stress due to DMBA exposure. However, this weakness has been anticipated by the existence of the standard and solvent control groups.

5. Conclusion

CXBCH equivalent doses of 5, 10, and 15 ml/70kgBW had a chemopreventive effect in SD rats induced with DMBA at 10x20 mg/kgBW. The chemopreventive mechanism of CXBCH is as a blocking agent by blocking the initiation process by inhibiting the carcinogenesis process.

CXBC antioxidant activity and mechanism decrease serum NO levels and increase liver and spleen GST enzyme activity. As an immunomodulator, CXBCH preparations increase the number of CD4, CD8, and CD4CD25 lymphocytes.

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25 November 2022

To whom it may concern

I hereby declare that the article stated below has undergone a proofreading process for grammatical and lexical errors. The work involved checking and correcting the use of tenses, punctuation, active/passive voices, subject-verb agreement, spelling, word appropriacy (choice and form), and wordiness. The work, however, did not involve any aspect related to content accuracy, validity, reliability, and clarity which shall be the full responsibility of the article authors.

Title:
Herbal honey preparations of curcuma xanthorriza and black cumin inhibit carcinogenesis through antioxidant and immunomodulatory activities in Sprague Dawley (SD) rats induced with Dimethylbenz(a)anthracene

Author(s):

1. Titiek Hidayati
2. Indrayanti
3. Endang Darmawan
4. Akrom

I further declare that the information provided in this letter is true. Any inquiries about the accuracy and precision of the proofreading work shall be addressed to ahmadmahgfur@gmail.com.

Yours faithfully,

Ahmad Mahgfur
Translator and Editor

Dear Reviewer

We appreciate your insightful and motivating comments and recommendations for strengthening our manuscript.

Our draft paper has been amended to reflect your views and ideas. A draft of our essay has been sent to a qualified linguist. The certificate of proofreading is included.

The attached point-by-point revision list.

Thank you

Best regard

Titiek and Akrom

comment and Feedback from Reviewer	Answer and Correction
many important publications that should be cited were missing. For example, - DMBA is converted to DMBA-3,4-diol-1,2-epoxide by cytochrome P450 enzymes 1A1 or 1B1 (CYP1A1 or CYP1B1) and microsomal hydrolase enzymes (DMBA-DE). (Line 44) DMBA-DE is a genotoxic and immunosuppressive active metabolite of DMBA. (Line 46)	Thank you for the advice and remarks. We are aware that the draft currently lacks numerous references that we have found. A few references have been included in accordance with the citation. When we changed it, the number of references increased from 60 to 96.
2) the average amounts of DMBA existed in the cigarette smoke and vehicle engine fumes should be added in the introduction section.	Information about the PAH content of cigarettes and the concentrations of benz anthracene in exhaust gases from moving vehicles has been included.(line 43 to line 52)
3) what are the difference between “moderate amounts (Line 48)” and “excessive amounts (Line 50)”?	Thanks for the comments. “Moderate amounts” is physiologically or normal value. We've corrected "moderate amounts" to "normal value or physiologically".(line 60-61)
4) In many places, the manuscript is written in a weak English language. This manuscript is not reached for evaluation.	Thank you for the comments. We have submitted articles for proof reading to professional editors.
5) what is “WT” meaning? (Line 52)	Thank “WT mice” =”wild tipe (WT) mice” (line 64)
6) what is “active radicals” meaning? (Line 84) Are there “inactive radicals”?	Thanks, thanks for the comments. We mean “Active radicals” is “free radicals”. We've fixed it. (line 96)
7) detail information about “the traditional herbal medicine industry certified by the Food and Drug Supervisory Agency (Line 112)” should be added.	Thanks "The traditional herbal medicine industry certified by the drug supervisory agency (FDSA)". FDSA has granted a certificate for good manufacturing practice (GMP) to CV Al Afiat. CV Al Afiat is a form of Small Business Traditional Medicine (Jamu) in Indonesia.(line 203-204)
8) who is the “experts”? (Line 114)	Prof. Dr. Subagus Wahyuono, Apt. An expert in pharmaceutical biology from the Faculty of Pharmacy, Gadjah Mada University. (line 126)

9) where did the authors obtain T47D cells used in this study? (Line 122)	We got cancer cell line (T47D, and Hela cells thanks to Prof. Dr. Edy Meianto, Apt. from The "Cancer and Chemoprevention research Center" Gadjah Mada University. (line 137-138)
10) why did the authors used female animals? (Line 124)	Thanks for the comments. In accordance with earlier studies, we used female test animals to undertake a chemopreventive test of the DMBA chemical carcinogen model. Female Sprague Dawley rats that are four to six weeks old have shown that DMBA causes cancer. (line 147-148)
11) what was the "EAPU"? (Line 126)	Thanks for the correction. We have corrected the draft, "We used 80 female Sprague Dawley rats aged 24 – 30 days with an average weight of 80-120 g obtained from the Preclinical and Experimental Animal Development Unit (PEADU), Gadjah Mada University." PEADU is a unit providing experimental animals from Gadjah Mada University." (line 139-142)
12) detail information about animal housing room, including temperature, humidity and light/dark cycle should be added. (Line 128).	80 – 120 g female Sprague Dawley rats were purchased from the Preclinical Experiment and Animal Development Unit (PEADU), Gadjah Mada University, Yogyakarta, Indonesia, when they were between four and six weeks old. The animals were kept in standardized climatic settings (22–28°C, 60–70% relative humidity, and a 12-hour cycle of darkness and light). They were kept in properly ventilated cages and given access to unlimited amounts of water as well as pelleted food (brailer-II, Japfa Comfeed Ltd). All animal experiments were conducted in accordance with the guidelines established by Universitas Ahmad Dahlan's ethical research committee. (line 149-153)
13) detail composition of diet used in this study should be added (Line 128).	Thank you Japfa Comfeed Ltd.'s standard feed was ordered. Rats are often fed on brailler-II pellets (BR-II), which are made from a

	combination of corn, soybean meal, wheat germ, coconut meal, fish meal, meat meal, rice flour, tapioca, and premixes of coconut oil and fish oil. (line 154-157)
14) detail information about company, such as Ohaus and Cosmos, should be added. (Line 131)	Thanks for the comments and feed back. We have added the company of each research tool and material (line 159-199)
15) the authors stated that "The quantities of thymoquinone, curcumin, polyphenols, and flavonoids in CX extract, BC extract, and CXBC preparations were determined using thymoquinone (Sigma), curcumin (Sigma), gallic acid (Sigma), and rutin (Sigma) standards. (Line 135)." Detail information about how to determine these compounds must be added.	Thank you for the comments and feedback. We write an explanation regarding the assay procedure in the inspection sub-procedure. (line 210-287)
16) what is the "predetermined composition" meaning? (Line 173)	Thank you The meaning "the predetermined composition"is composition formulation of CXBCH preparation (line 207)
17) what is the "certain speed" meaning? (Line 174)	'constant speed" (line 208)
18) where did the authors obtain the "Folin-Ciocalteau reagent"? (Line 179)	Folin-Ciocalteu reagent (Merck, Germany) (line 220)
19) the authors stated that "1 mL of 50,000 ppm sample solution was pipetted and placed in a 10 mL volumetric flask (Line 191)." Here, 50,000 ppm is 5g/100mL (5%). Is this the correct information? The sample was dissolved as concentration of 5g/100mL?	Thank you We've revised the draft. We prepared a sample stock solution of the test preparation with a concentration of 5%. 500 mg of sample was put in a 10 mL volumetric flask then added with aqua until it reached the limit of 10 mL and then homogenized. (line 231-239)

20) the authors stated that "then quantified using a UV-Visible spectrophotometer at a wavelength of 200 – 400 nm. (Line 203)" How to analyze the amounts using such range of wavelength?	"then quantified using a UV-Visible spectrophotometer at a wavelength of 200 – 400 nm to find out the specific wave length number of thymoquinone" (line 247-249)
21) the authors stated "Calibration curves were made using a series of reference standard solutions with five different concentrations (0.5, 1, 2, 5, and 10 g/mL). (Line 218)" Is this the correct information? In my opinion, it is impossible to prepare the standard solution with such a higher concertation (10 g/mL).	It should be 0.5, 1.0, 2.0, 5.0, and 10.0 microgram/mL (line 255, 264)
22) the authors stated that "T47D cells were cultured in 2% FBM supplemented with 1% penicillin (100 units/mL) and streptomycin (100 g/mL) at 37 °C, 5% CO2 in an incubator. (Line 247)" It might be impossible to use such concentration (100g/mL) of streptomycin.	"T47D cells were cultured in 2% FBM supplemented with 1% penicillin (100 units/mL) and streptomycin (100 ug/mL) at 37 °C, 5% CO2 in an incubator.(line 305-309)
23) the authors stated that "A 200 L of DMSO reagent was added to dissolve the formazan product in each well (Line 253)." I cannot understand, why the authors added such huge amount of DMSO (200L) in the well.	"A 200 uL of DMSO reagent was added to dissolve the formazan product in each well (line 313)
24) the authors stated that "Group II was given CXBC1 (equivalent to 1x5 ml/70kg BW). (Line 260)," indicating that the treatment amount was 0.07 mL/kg body weight. In this study, the body weight of rats were a few 100 g. In my opinion, it is quite difficult to inject such low amount (about 10 uL/animal).	Many thanks We changed the provided dose from a 70kg human dose to a dose for rats (200 grams): CXBCH1=0.018 x 5 ml= 0.09 ml CXBC CXBCH2=0.018 x 10 ml= 0.18 ml CXBC CXBCH3=0.018 x 15 ml =0.27 ml CXBC

	To create a CXBC preparation solution that comprises 0.09 ml of CXBC/1 ml of volum solution, we diluted the CXBC preparation using aqua. 100 ml of aqua were added after 1 ml of CXBC, and the mixture was then agitated until it was homogenous. Because each set of test animals weighed 100 grams, a solution comprising 0.09 mL/1 mL of CXBC was administered along with volumes of 1/2 mL, 1 mL, and 1.5 mL of CXBCH1, CXBCH2, and CXBCH3. (Line 321-327)
25) detail information about "Integrated Testing and Examination Institute unit I (Line 283)" should be added.	Experimental Animal Service Unit of Integrated Testing and Examination Institute of Gadjah Mada University, Indonesia (line 346-347)
26) detail information about "Sysmeix kx 12 hematology analyzer (Line 285)" should be added.	hematology analyzer (Sysmex kx 12, Sysmex Ltd. Indonesia) (line 348)
27) what kind of anesthesia was used sacrifice?	the rats were sacrificed using chloroform vapor. (line 374)
29) "rpm" should be "g (gravity)" for example, Line 292.	1,789 g (line 355)
30) the error bar was missing on Figure 2.	We've added an error bar (line 553-570)
31) detail data about body weight and food intake during the experiments must be added	Animal body weight 100-300 g. The animal food was BR2 (Japfa Comfeed Ltd.) (line 154-157)

Dear Reviewer

We appreciate your insightful and motivating comments and recommendations for strengthening our manuscript.

Our draft paper has been amended to reflect your views and ideas. The attached point-by-point revision list.

A draft of our essay has been sent to a qualified linguist. The certificate of proofreading is included.

Best Regard

Titiek Hidayati and Akrom

No	Comments	Answer
1	In the current work, the authors examined the anticancer activity in vitro in a single cell line (T47D cells; mammary cancer cell line). This is not adequate. The authors are advised to examine the cytotoxic activity on 2 additional cancer cell lines to lend more reliability to the data. **This point needs to be carefully addressed by the authors.**	We followed up on new data from cancer cell viability testing (T47D, Hela, and HTB-183) with 72-hour exposure to test materials (CXBCH), as recommended, and reviewer comments. (line 553-572)
2	Regarding the Curcuma xanthorrhiza (CX) extract, black cumin seed extract (BC) extract, and the mixture extract (CXBC), the authors are advised to show all the chemical charts (e.g., HPLC, NMR, ….) that confirm the identity of all ingredients. (not just as a total of flavonoids, alkaloids, etc). Without these data, the results of the study cannot be reliable. **This point needs to be carefully addressed by the authors**	On the findings of the GCMS and LC-HRMS tests performed on CXBC preparations, new information has been included. (line 512-537)
3	The reviewer has doubt about the stability of the used mixture of honey, Curcuma xanthorrhiza (CX) extract, and black cumin seed extract (BC) extract. In fact, the presence of seed oil in the mixture would make it difficult for the mixture to stay as is. Did the authors convert the mixture into a stable emulsion? Please, elaborate on this point in the material and methods section.	CXE and BCE are combined with honey solvent to create CXBC herbal honey. We used a mixture as an emulsifier to help the CX extract blend with the BC in the honey. The process of creating honey is summarized as follows: When we first prepare honey as a solvent, we weigh it according to our needs before adding tragacanth up to 5–10% of the weight of the honey as an emulsifier. Tragacanth was previously dissolved in water, which was then gradually added to the honey while stirring. All the ingredients, which have been weighed in accordance with the formula, are then gradually added to the honey, which is being swirled with a blower, after the tragacanth has been incorporated into it. (line 205-215)
4	In section 2.4. (cytotoxicity test). Why was incubation time (24 h) used in the manuscript? Treatment for only 24 h seems not able to give enough picture of the anti-cancer effect of candidate agents. In general,	In accordance with the testing protocol, the test substance was incubated in cell culture for 72 hours in our facility. We have examined the viability of cancer cells T47D, Hela, and HTB-183 in accordance with the suggestions. (line 311-325)

	a time course covering up to 24 or 72 h should be expected.	
5	Why did the authors choose to examine the cytotoxicity using MTT assay and not test the antiproliferative assay such as using the propidium iodide assay? In fact, the MTT assay is a test and does not give much information about cell death but about viability. In MTT assay, false positive results may be encountered because the cells can stop proliferation and metabolism but are still alive and are not dead. However, when studying anticancer effect of drugs, what basically we are looking is the antiproliferative effect rather than actual cell-killing. Hence, candidate assays such as propidium iodide can give more reliable data.	We employ the MTT method in reference to previous researchers. However, after reading the reviewer comments and the meta-analysis results, we realized the shortcomings of the MTT method. To mitigate the MTT method's weakness, we present the data from the viability examination results in two ways: 1. as a graph with the mean difference test between groups, and 2. as IC50 data presentation. We also add information on CXBC's chemopreventive mechanism via its effect on p53 and Caspase-3 expression in Hela cells. (line 323-324)
6	The authors are advised to dig more into the potential mechanisms involved in the cytotoxic actions observed including the cell cycle analysis to confirm whether apoptosis occurred in the T47D cancer cell line	We employ the MTT method in reference to previous researchers. By observing the effect of CXBC preparations 1/2 and IC50 concentrations on p53 and caspase-3 in Hela cells, we added data on the chemopreventive mechanism of CXBCH preparations.
7	Likewise, the authors are advised to examine apoptosis-related mechanisms such as the activity of caspase-3, 7, and 9 alongside the protein expression of Bax and Bcl2.	We employ the MTT method in reference to previous researchers. By observing the effect of CXBC preparations 1/2 and IC50 concentrations on p53 and caspase-3 in Hela cells, we added data on the chemopreventive mechanism of CXBCH preparations.
8	In figure 2, The authors are advised to show the original curves for the viability of T47D cells when treated with different mixtures, not just presenting the IC50. Why did not the authors show the IC50 of honey similar to what they did for CX extract and BC extract?	According to the research objectives, we added viability graphs for three different cell types—namely, T47D, Hela, and HTB-183 cells—but we concentrated on evaluating CXBCH preparations and omitted CXE, BCE, or honey.
9	The statistical data analysis in tables 3 and 4 is inappropriate. The authors are advised to show the statistical significance (if any) between the	Thank you for your suggestions and feedback. We revised the manuscript in response to your suggestions. Thank you very much once more. (line 625-641)

	normal vs DMBA gp, and more importantly between DMBA versus CXBC groups or thymoquinone. In fact, showing statistical significance between groups and the normal reference range is not acceptable.	
10	In lines 405-406, the authors state "the mean difference between groups was determined, and then the Anava and Tukey (LSD) tests were conducted with a significance level of $p < 0.05$". The name of the statistical analysis test is one-way ANOVA (not anava). The LSD is not the abbreviation of the post-hoc test Tukey. In fact, LSD stands for least significant difference" test which is another statistical analysis test. Please, re-write carefully.	We appreciate the feedback and ideas. In response to your recommendations, We revised. (line 481-498)
11	In the statistical analysis section, did the authors check data normality and homogeneity before proceeding to one-way ANOVA? **Authors are advised to address this point and add the answers to the comment in the material and methods section**.	I appreciate your advice. We revised the manuscript based on your feedback. Thank you very much once more. Body weight and laboratory animal data were subjected to normality and homogeneity tests. According to these findings, body weight data meets the requirements for parametric data, whereas the majority of laboratory data (hemogram profile, blood chemistry, NO levels, GST levels, CD4, CD8, and CD4CD25 cell counts) do not. Kruskal Wallis was then used to test for mean differences between groups in laboratory data, while a repeat measure test was used to test for differences in mean body weight between groups and measurement times.
12	In figure 4, the authors are advised to show all the histopathological changes and denote them with different types of arrows. As it	I appreciate your advice. The subtitles and explanations for the histology images have been enhanced. (line 699-710)

	currently stands, the figure is not informative.	
13	The numbers shown in tables 3, 4 5, 6, and 7 are in the wrong format. For example, the numbers are written in table 7 as 0,20 ± 0,70. Instead, it should be written as 0.20 ± 0.70.	I appreciate the correction. The way the numbers were written in the script has been corrected.
14	In section 2.4., the authors are advised to describe the rationale for using the T47D mammary cancer cell line for testing the anticancer potential of tested agents.	A viability test for the impact of the CXBCH preparation on the three cancer cells T47D, Hela, and HTB-183 has been added. Hela is a cell line for cervical cancer, HTB-183 is a cell line for non-small cell lung cancer, and T47D is a cell line for breast cancer. The three different cell types stand for the three different cancers that are most frequently encountered worldwide. All three forms of cancer are at danger due to cigarette smoke exposure.
15	There are several typos in the current manuscript. For example, in line 253, the authors state "A 200 L of DMSO reagent was added to dissolve the formazan product in each 253well". The unit needs to be modified to 200 µL!	I appreciate the correction. The script's writing has been updated. (line 319-320)
16	The authors are advised to use "dimethylbenz(a)anthracene" instead of "dimethylbenz anthracene" in the title since it is more commonly used in the literature. Please address this issue in the entire manuscript.	I appreciate your advice. All DMBA spellings have been changed to dimethylbenz(a)anthracene.

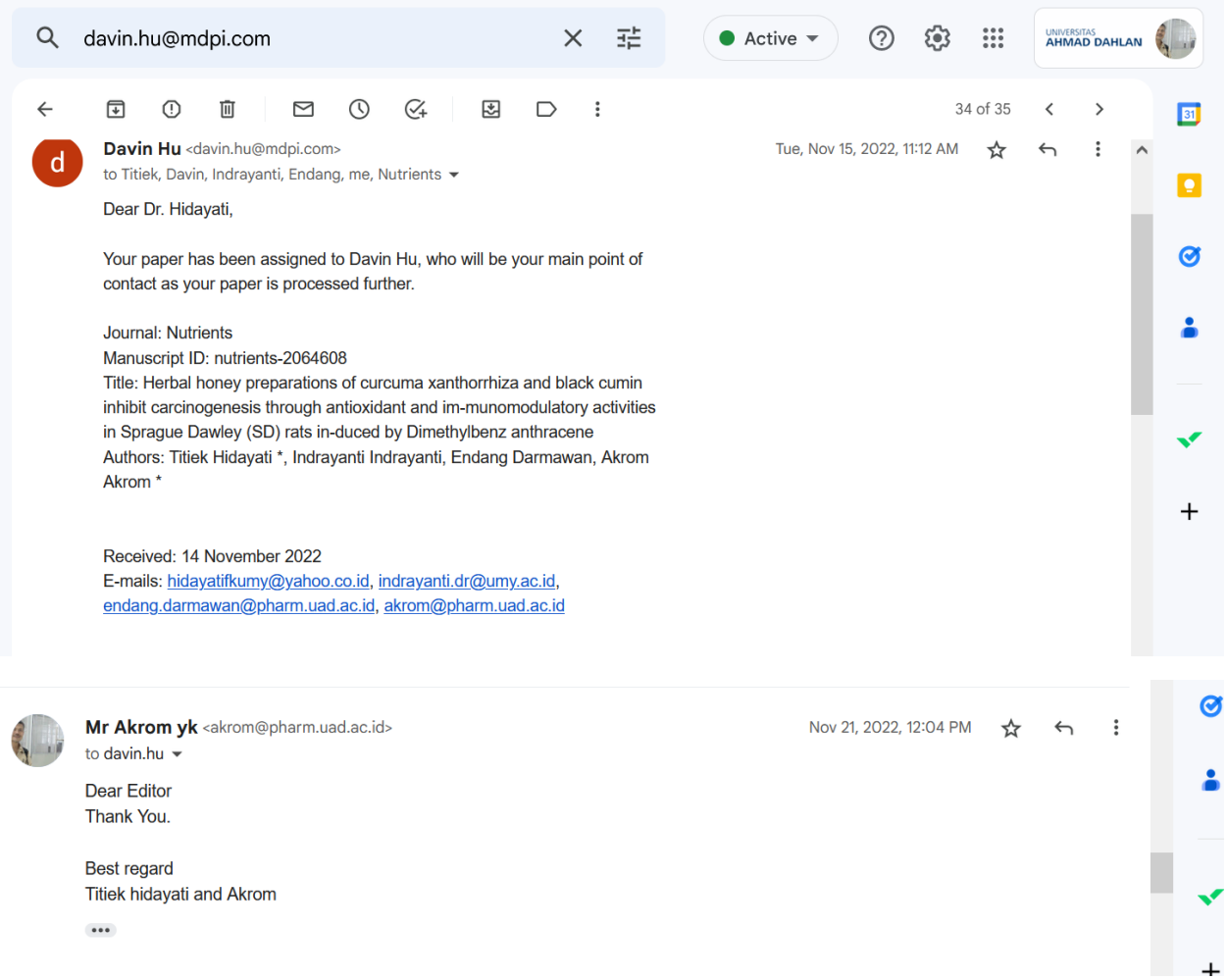
Dear Reviewer

We appreciate your insightful and motivating comments and recommendations for strengthening our manuscript.

Our draft paper has been amended to reflect your views and ideas, especially in the background section, method section (study design), addition of fresh data, and enhancements to the presentation and conclusion sections. The updated manuscript can be found here.

Best Regard

Titiek Hidayati and Akrom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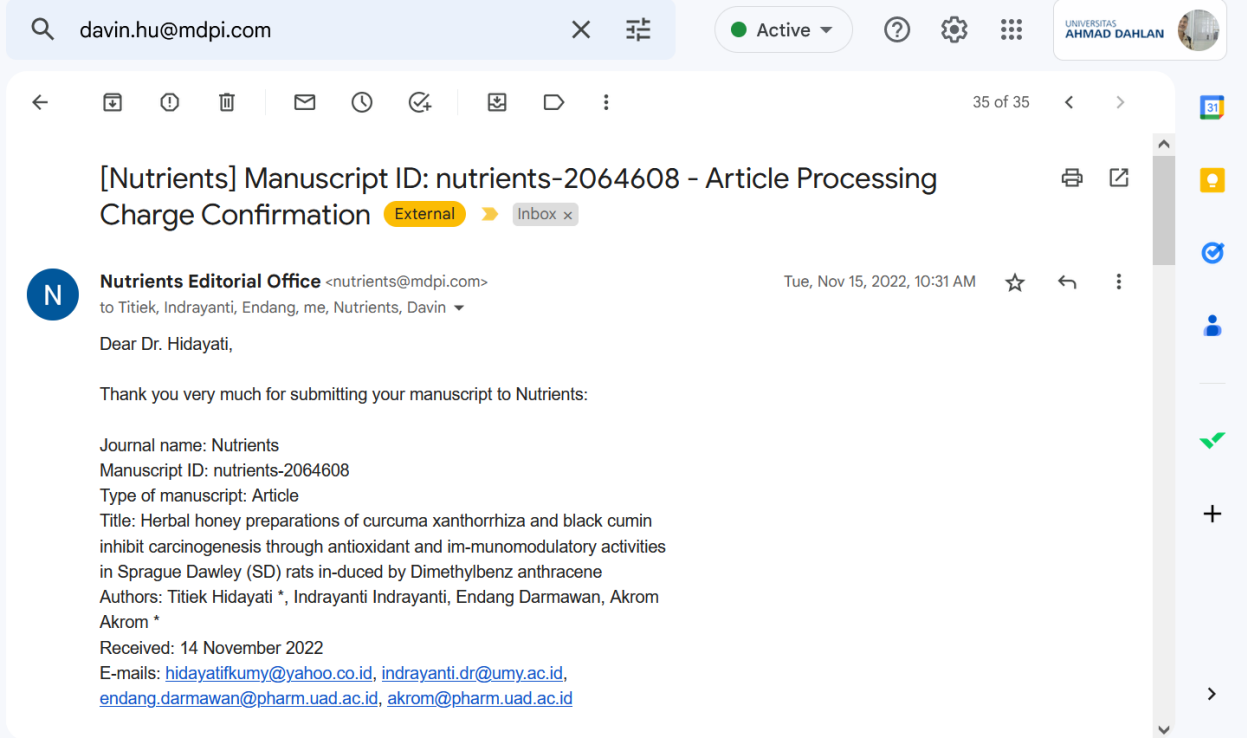
Davin Hu <davin.hu@mdpi.com>
to Titiek, Davin, Indrayanti, Endang, me, Nutrients
Tue, Nov 15, 2022, 11:12 AM
Dear Dr. Hidayati,
Your paper has been assigned to Davin Hu, who will be your main point of contact as your paper is processed further.
Journal: Nutrients
Manuscript ID: nutrients-2064608
Title: Herbal honey preparations of curcuma xanthorrhiza and black cumin inhibit carcinogenesis through antioxidant and im-munomodulatory activities in Sprague Dawley (SD) rats in-duced by Dimethylbenz anthracene
Authors: Titiek Hidayati *, Indrayanti Indrayanti, Endang Darmawan, Akrom Akrom *
Received: 14 November 2022
E-mails: hidayatifkumy@yahoo.co.id, indrayanti.dr@umy.ac.id, endang.darmawan@pharm.uad.ac.id, akrom@pharm.uad.ac.id

Mr Akrom yk <akrom@pharm.uad.ac.id>
to davin.hu
Nov 21, 2022, 12:04 PM
Dear Editor
Thank You.
Best regard
Titiek hidayati and Akrom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[Nutrients] Manuscript ID: nutrients-2064608 - Article Processing Charge Confirmation
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Nutrients Editorial Office <nutrients@mdpi.com>
to Titiek, Indrayanti, Endang, me, Nutrients, Davin
Tue, Nov 15, 2022, 10:31 AM
Dear Dr. Hidayati,
Thank you very much for submitting your manuscript to Nutrients:
Journal name: Nutrients
Manuscript ID: nutrients-2064608
Type of manuscript: Article
Title: Herbal honey preparations of curcuma xanthorrhiza and black cumin inhibit carcinogenesis through antioxidant and im-munomodulatory activities in Sprague Dawley (SD) rats in-duced by Dimethylbenz anthracene
Authors: Titiek Hidayati *, Indrayanti Indrayanti, Endang Darmawan, Akrom Akrom *
Received: 14 November 2022
E-mails: hidayatifkumy@yahoo.co.id, indrayanti.dr@umy.ac.id, endang.darmawan@pharm.uad.ac.id, akrom@pharm.uad.ac.id

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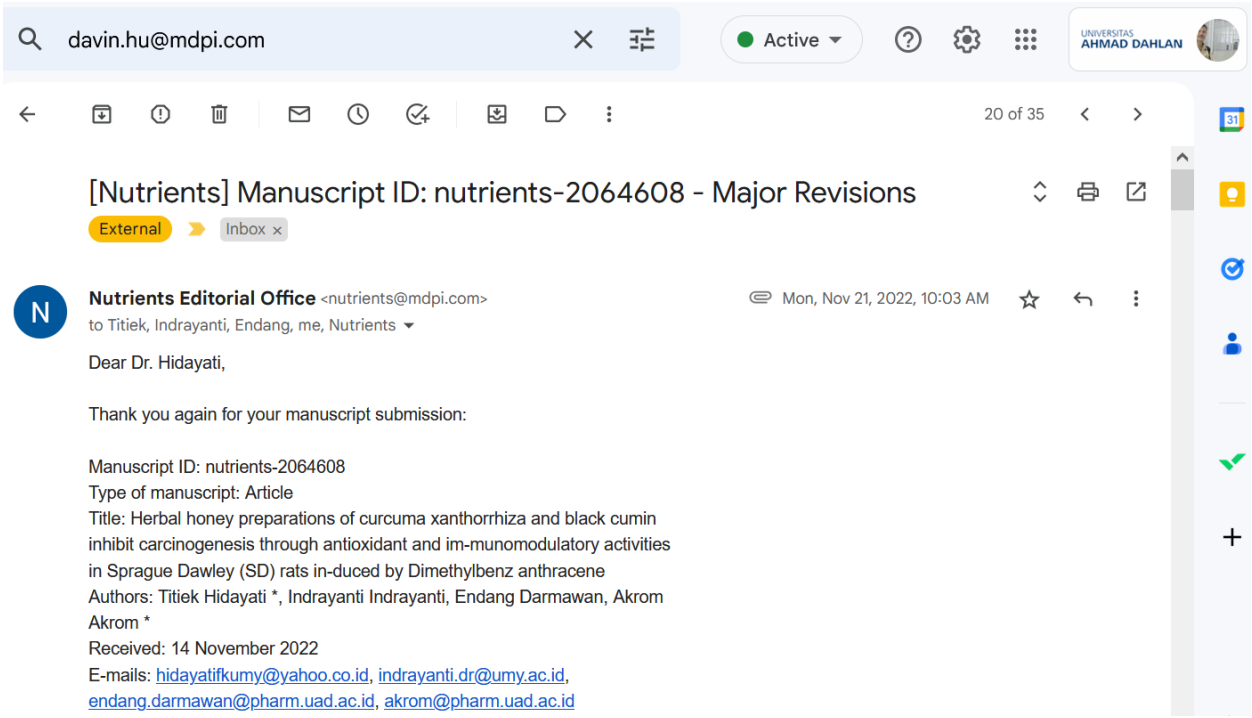
Please also check and confirm that the below information for the invoice address is correct:

Name: Titiek - Hidayati
Address: Dr. Titiek - Hidayati
Universitas Muhammadiyah Yogyakarta
Public Health and Family medicine Department, Medicine and Health Science Faculty
Kapten Tendean no 59 kota madya Yogyakarta



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Please use the version of your manuscript found at the *attached* for your revisions.

(I) Please check that all references are relevant to the contents of the manuscript.
(II) Any revisions to the manuscript should be marked up using the *highlight* function if you are using MS Word/LaTeX, such that any changes can be easily viewed by the editors and reviewers.
(III) Please provide a cover letter to explain, *point by point*, the details of the revisions to the manuscript and your responses to the referees' comments.
(IV) If you found it impossible to address certain comments in the review reports, please include an explanation in your appeal.
(V) The revised version will be sent to the editors and reviewers.

If one of the referees has suggested that your manuscript should undergo extensive English revisions, please address this issue during revision. We propose that you use one of the editing services listed at https://www.mdpi.com/authors/english or have your manuscript checked by a native English-speaking colleague.

Do not hesitate to contact us if you have any questions regarding the revision of your manuscript. We look forward to hearing from you soon.

Kind regards,
Mr. Davin Hu
E-Mail: davin.hu@mdpi.com

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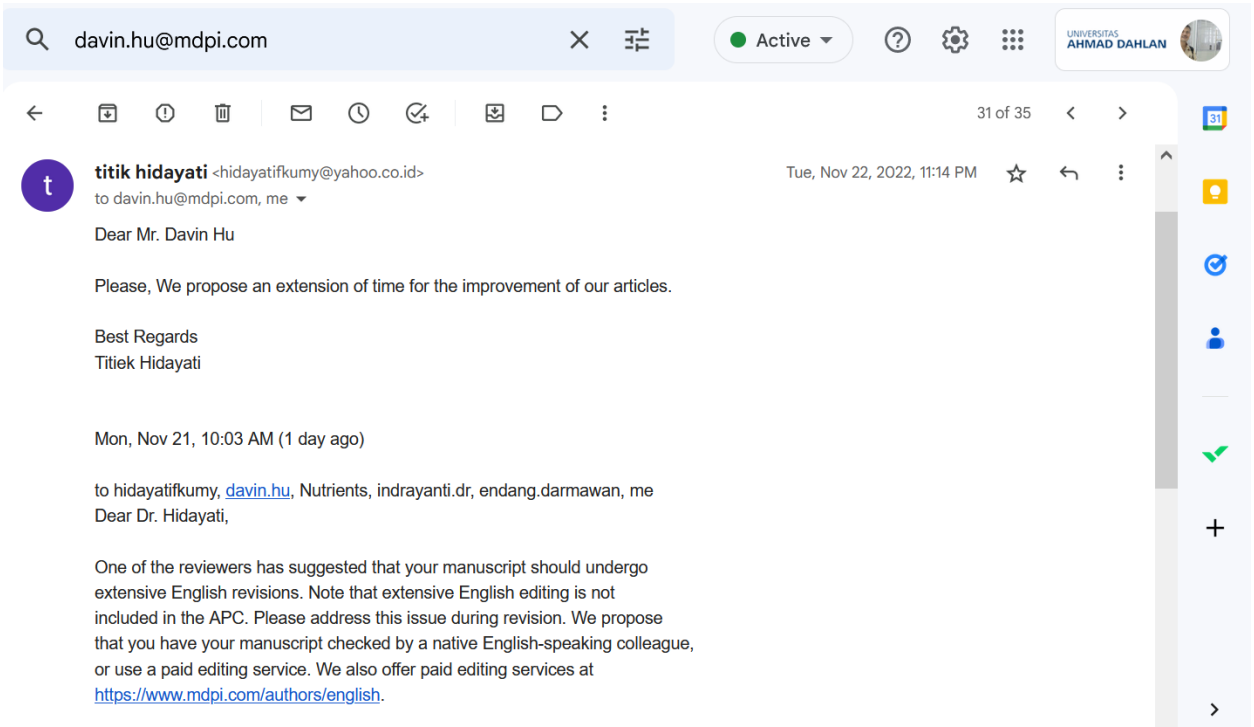


Mr Akrom yk <akrom@pharm.uad.ac.id>
to davin.hu

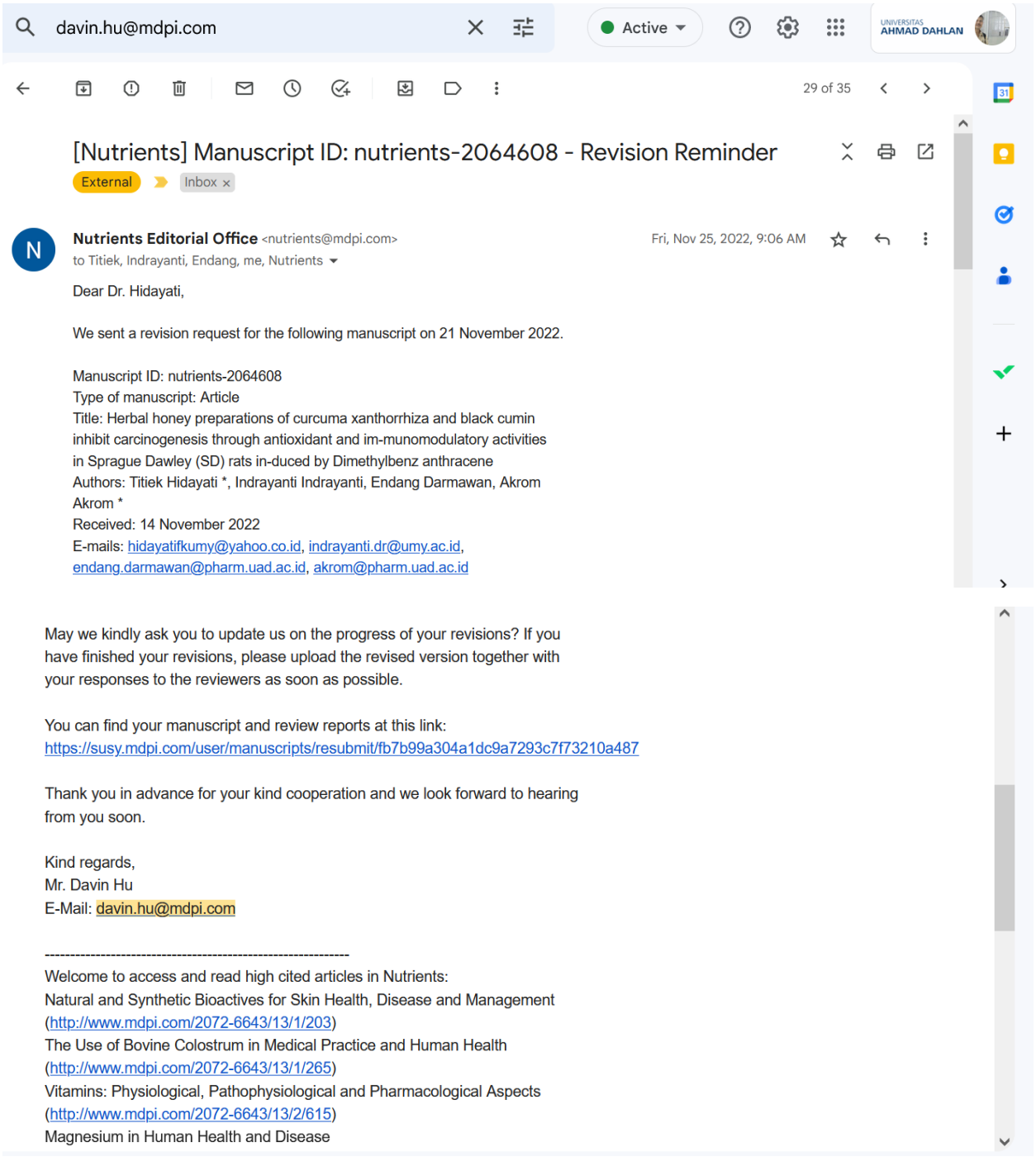
Tue, Nov 22, 2022, 11:17 PM

Dear Mr. Davin Hu

Please, We propose an extension of time for the improvement of our articles.

Best Regards

Permintaan revisi



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[Nutrients] Manuscript ID: nutrients-2064608 - Revision Reminder

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Nutrients Editorial Office <nutrients@mdpi.com>
to Titiek, Indrayanti, Endang, me, Nutrients

Fri, Nov 25, 2022, 9:06 AM

Dear Dr. Hidayati,

We sent a revision request for the following manuscript on 21 November 2022.

Manuscript ID: nutrients-2064608
Type of manuscript: Article
Title: Herbal honey preparations of curcuma xanthorrhiza and black cumin inhibit carcinogenesis through antioxidant and im-munomodulatory activities in Sprague Dawley (SD) rats in-duced by Dimethylbenz anthracene
Authors: Titiek Hidayati *, Indrayanti Indrayanti, Endang Darmawan, Akrom Akrom *
Received: 14 November 2022
E-mails: hidayatifkumy@yahoo.co.id, indrayanti.dr@umy.ac.id, endang.darmawan@pharm.uad.ac.id, akrom@pharm.uad.ac.id

May we kindly ask you to update us on the progress of your revisions? If you have finished your revisions, please upload the revised version together with your responses to the reviewers as soon as possible.

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Magnesium in Human Health and Disease

Mr Akrom yk <akrom@pharm.uad.ac.id> Nov 25, 2022, 10:15 AM
to davin.hu

Dear Editor
Thank you, we are currently making revisions according to comments from reviewers. Thank you for your attention, since there are quite a lot of comments from reviewers and requires major revisions, would you like us to be given more time so that we can revise properly?

Best regard
Titiek H and Akrom

Reply Forward

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28 of 35

[Nutrients] Manuscript ID: nutrients-2064608 - Revision Reminder

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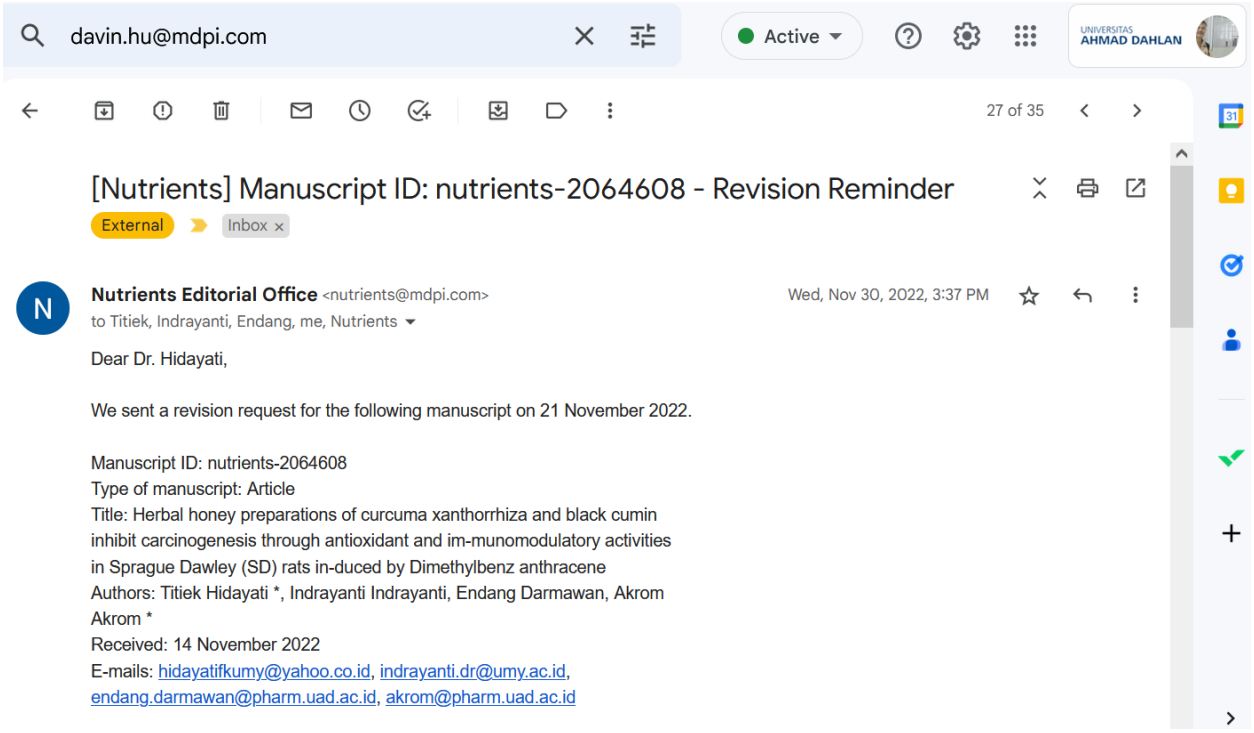
Nutrients Editorial Office <nutrients@mdpi.com> Mon, Nov 28, 2022, 11:15 AM
to Titiek, Indrayanti, Endang, me, Nutrients

Dear Dr. Hidayati,

We sent a revision request for the following manuscript on 21 November 2022.

Manuscript ID: nutrients-2064608
Type of manuscript: Article
Title: Herbal honey preparations of curcuma xanthorrhiza and black cumin inhibit carcinogenesis through antioxidant and im-munomodulatory activities in Sprague Dawley (SD) rats in-duced by Dimethylbenz anthracene
Authors: Titiek Hidayati *, Indrayanti Indrayanti, Endang Darmawan, Akrom Akrom *
Received: 14 November 2022
E-mails: hidayatifkumy@yahoo.co.id, indrayanti.dr@umy.ac.id, endang.darmawan@pharm.uad.ac.id, akrom@pharm.uad.ac.id



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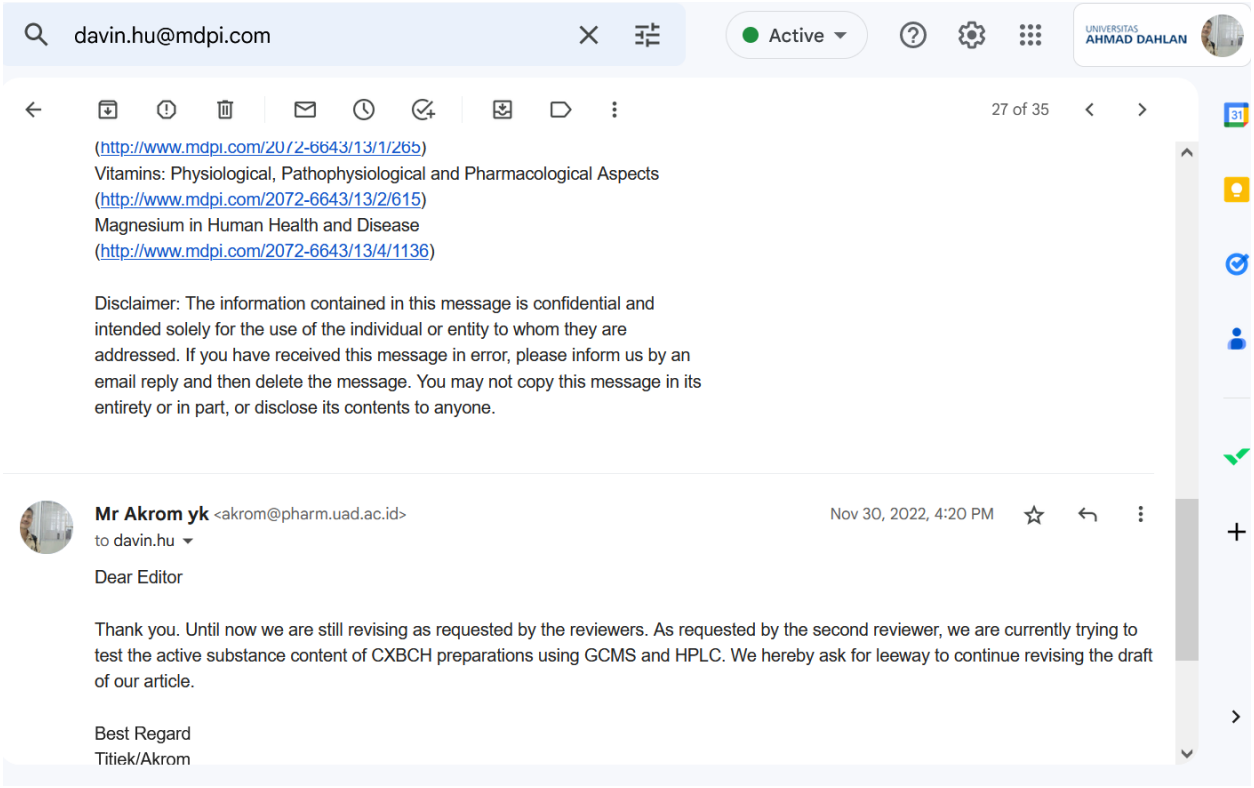
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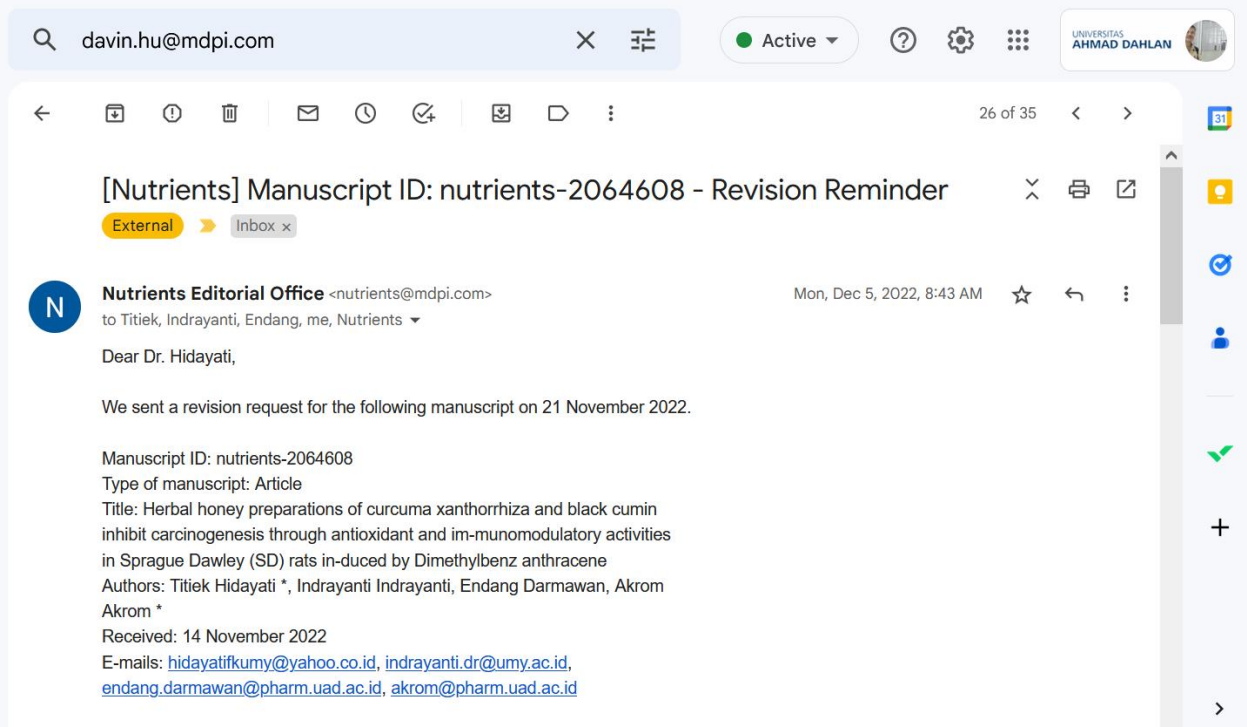
Mr Akrom yk <akrom@pharm.uad.ac.id>
to davin.hu
Nov 30, 2022, 4:20 PM
Dear Editor
Thank you. Until now we are still revising as requested by the reviewers. As requested by the second reviewer, we are currently trying to test the active substance content of CXBCH preparations using GCMS and HPLC. We hereby ask for leeway to continue revising the draft of our article.
Best Regard
Titiek/Akrom



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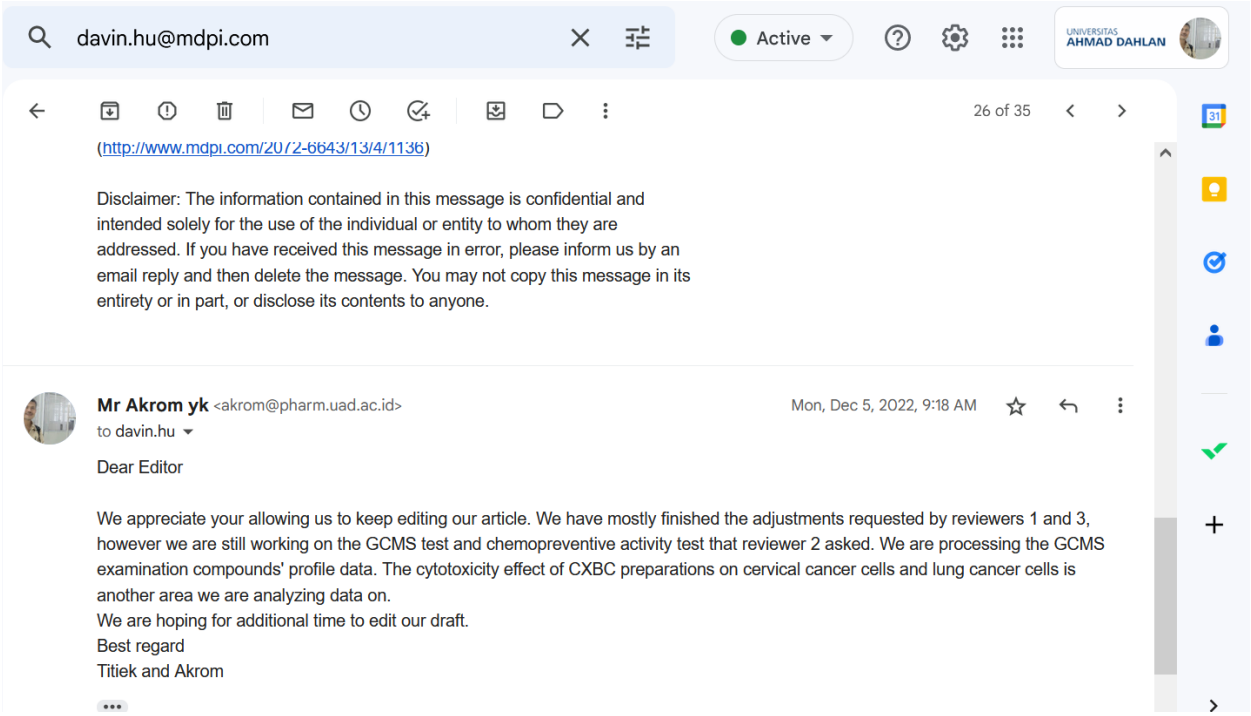
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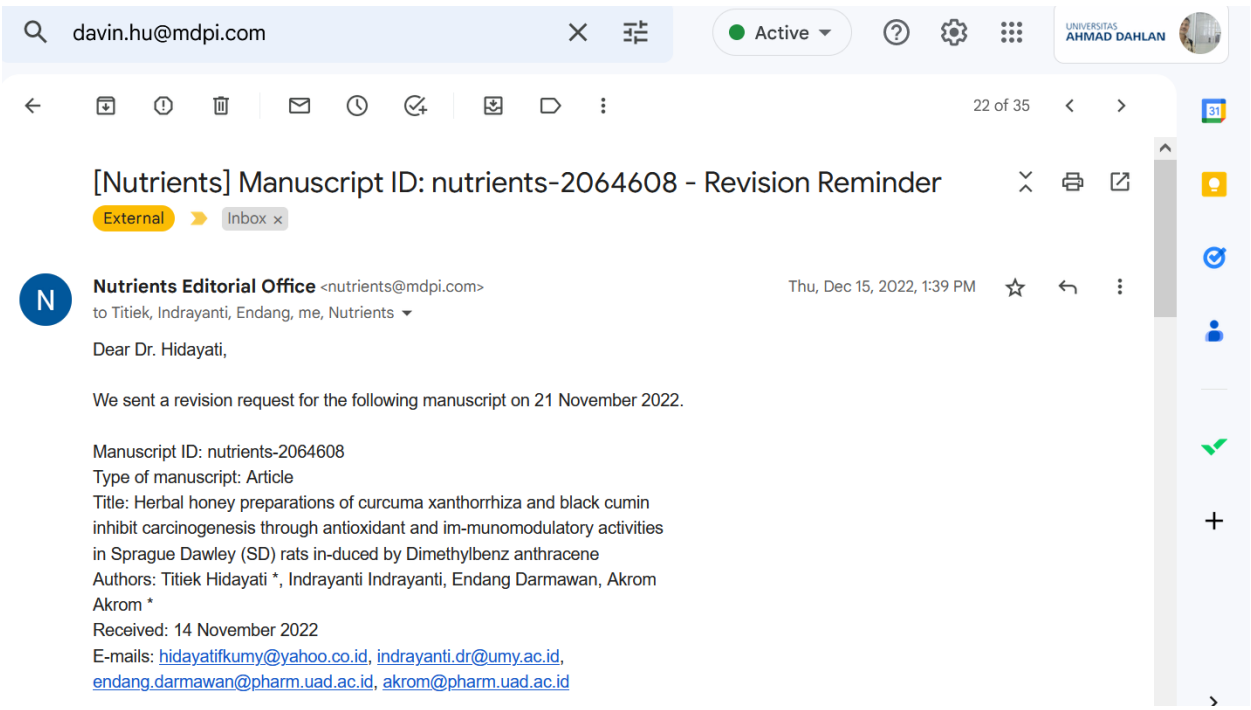
Mr Akrom yk <akrom@pharm.uad.ac.id>
to davin.hu
Mon, Dec 5, 2022, 9:18 AM
Dear Editor
We appreciate your allowing us to keep editing our article. We have mostly finished the adjustments requested by reviewers 1 and 3, however we are still working on the GCMS test and chemopreventive activity test that reviewer 2 asked. We are processing the GCMS examination compounds' profile data. The cytotoxicity effect of CXBC preparations on cervical cancer cells and lung cancer cells is another area we are analyzing data on.
We are hoping for additional time to edit our draft.
Best regard
Titiek and Akrom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[Nutrients] Manuscript ID: nutrients-2064608 - Revision Reminder
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to Titiek, Indrayanti, Endang, me, Nutrients
Thu, Dec 15, 2022, 1:39 PM
Dear Dr. Hidayati,
We sent a revision request for the following manuscript on 21 November 2022.
Manuscript ID: nutrients-2064608
Type of manuscript: Article
Title: Herbal honey preparations of curcuma xanthorrhiza and black cumin inhibit carcinogenesis through antioxidant and im-munomodulatory activities in Sprague Dawley (SD) rats in-duced by Dimethylbenz anthracene
Authors: Titiek Hidayati *, Indrayanti Indrayanti, Endang Darmawan, Akrom Akrom *
Received: 14 November 2022
E-mails: hidayatifkumy@yahoo.co.id, indrayanti.dr@umy.ac.id, endang.darmawan@pharm.uad.ac.id, akrom@pharm.uad.ac.id

May we kindly ask you to update us on the progress of your revisions? If you have finished your revisions, please upload the revised version together with your responses to the reviewers as soon as possible.

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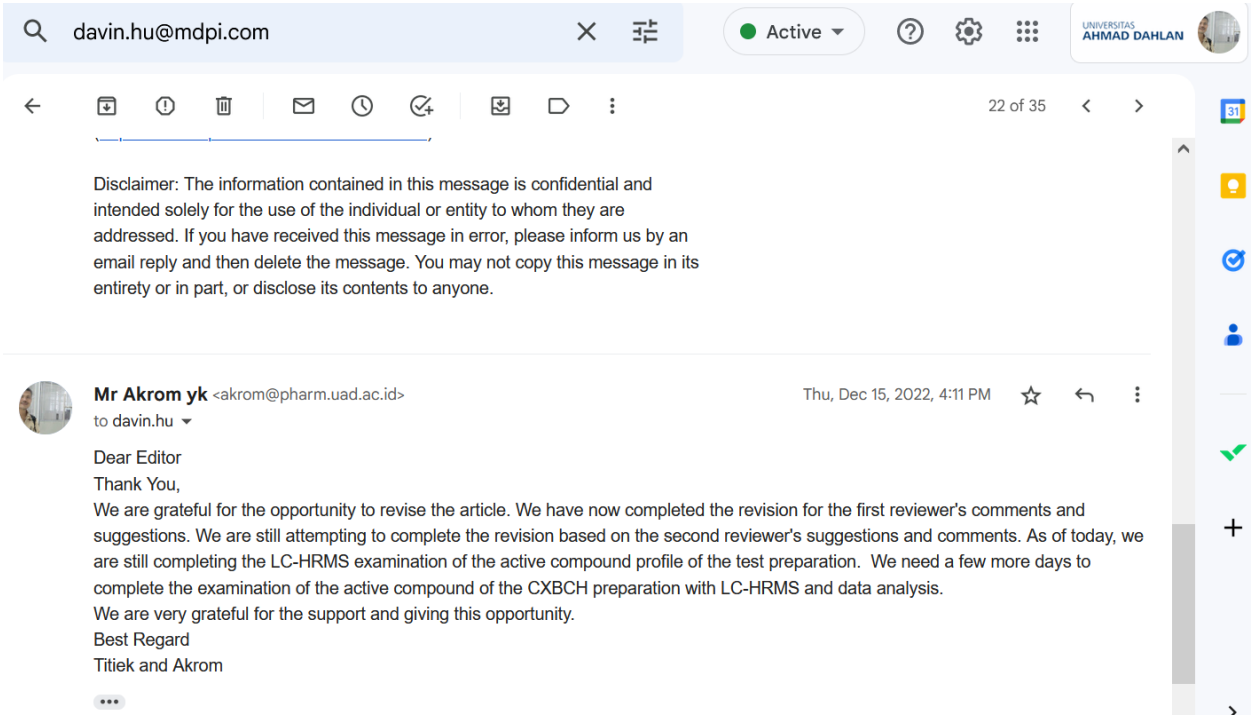
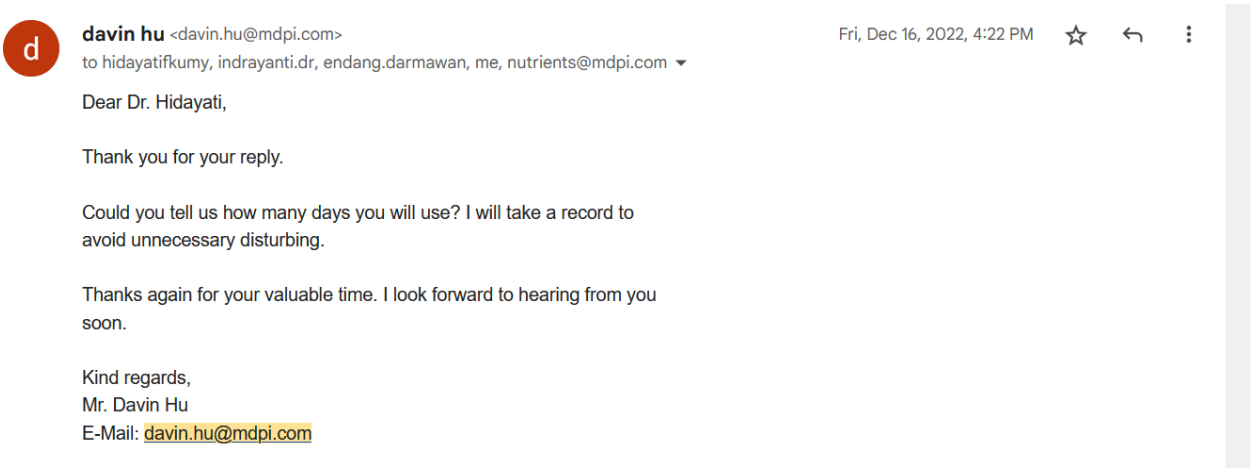
Magnesium in Human Health and Disease



Peringatan untuk revisi dan jawaban permohonan tambahan waktu



davin hu <davin.hu@mdpi.com> Fri, Dec 16, 2022, 4:22 PM

to hidayatifkumy, indrayanti.dr, endang.darmawan, me, nutrients@mdpi.com

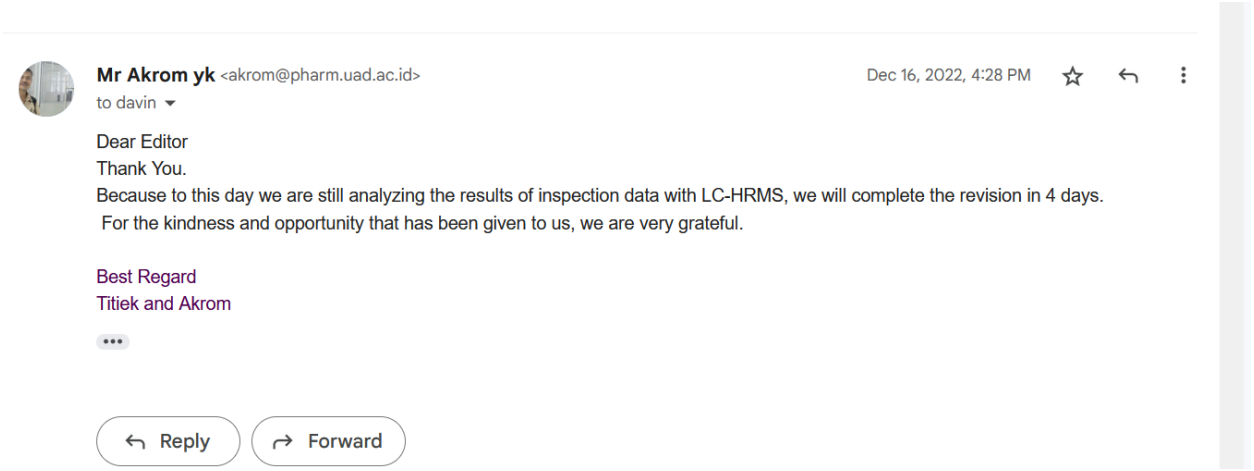
Dear Dr. Hidayati,

Thank you for your reply.

Could you tell us how many days you will use? I will take a record to avoid unnecessary disturbing.

Thanks again for your valuable time. I look forward to hearing from you soon.

Kind regards,
Mr. Davin Hu
E-Mail: davin.hu@mdpi.com



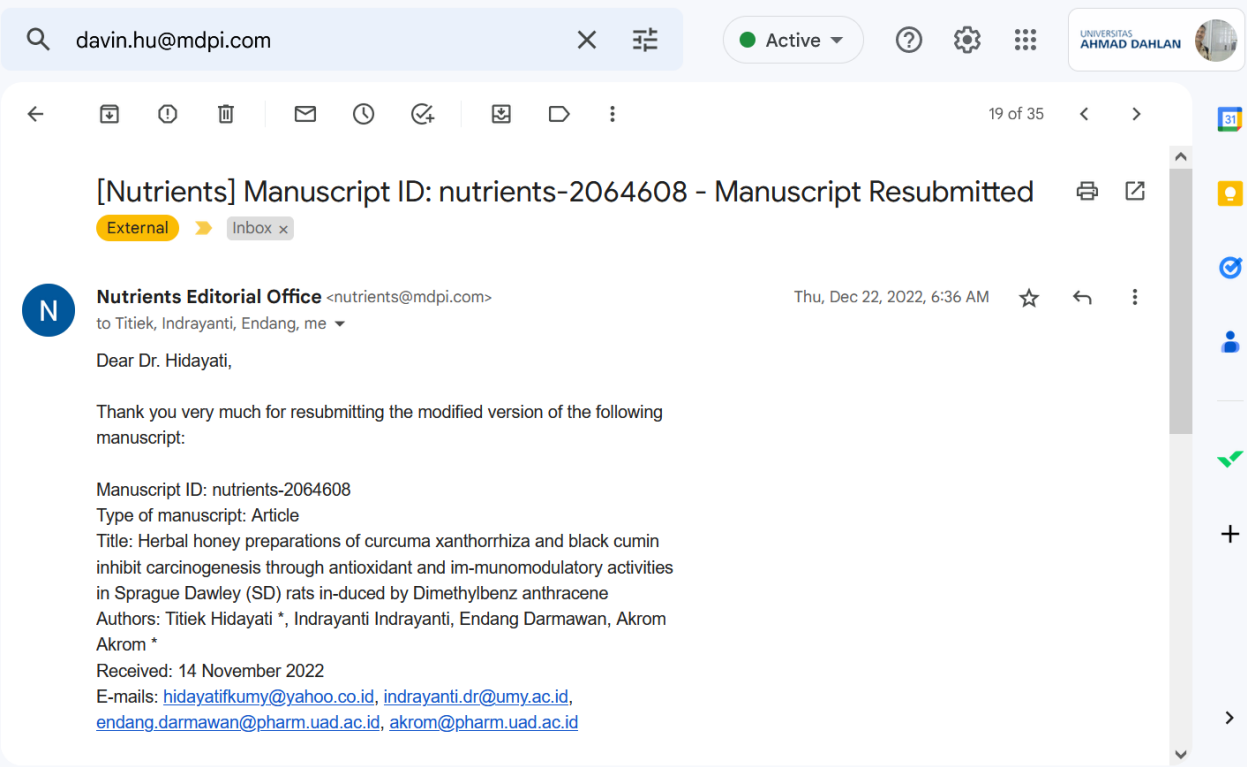
Mr Akrom yk <akrom@pharm.uad.ac.id> Dec 16, 2022, 4:28 PM

to davin

Dear Editor
Thank You.
Because to this day we are still analyzing the results of inspection data with LC-HRMS, we will complete the revision in 4 days.
For the kindness and opportunity that has been given to us, we are very grateful.

Best Regard
Titiek and Akrom

Reply Forward



Received: 14 November 2022
E-mails: hidayatifkumy@yahoo.co.id, indrayanti.dr@umy.ac.id, endang.darmawan@pharm.uad.ac.id, akrom@pharm.uad.ac.id

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A member of the editorial office will be in touch with you soon regarding progress of the manuscript.

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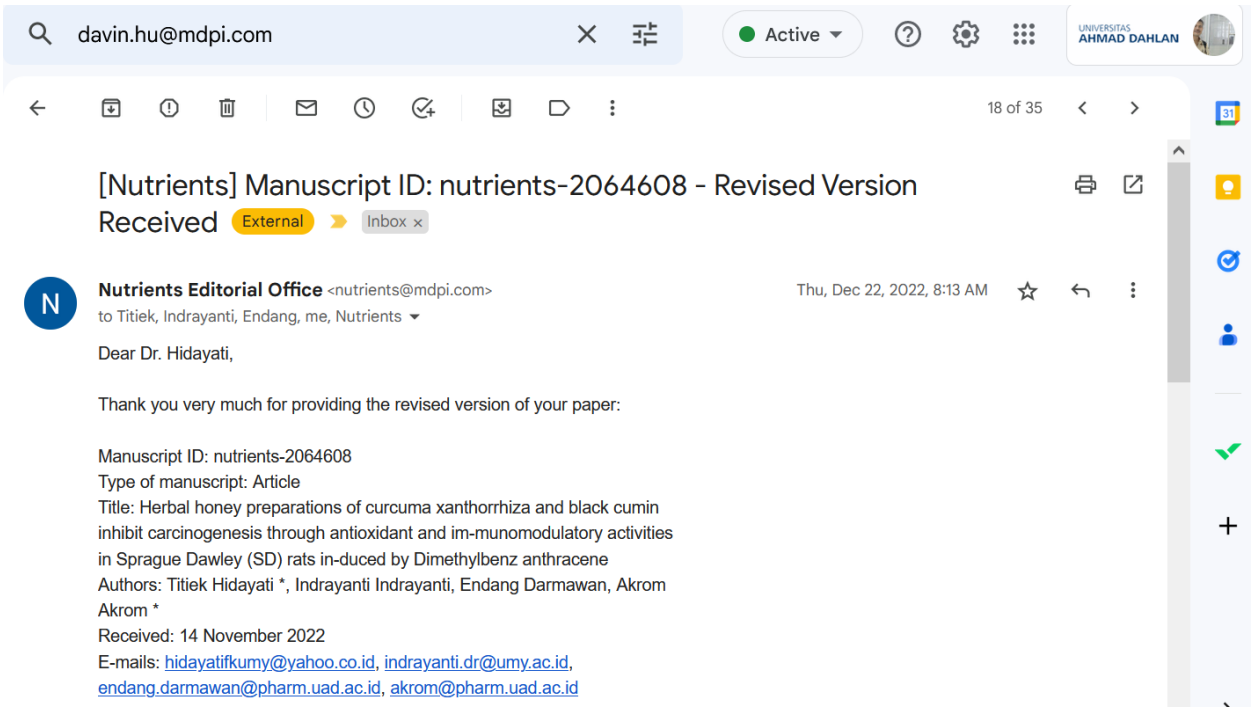

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18 of 35

[Nutrients] Manuscript ID: nutrients-2064608 - Revised Version Received

External Inbox ×

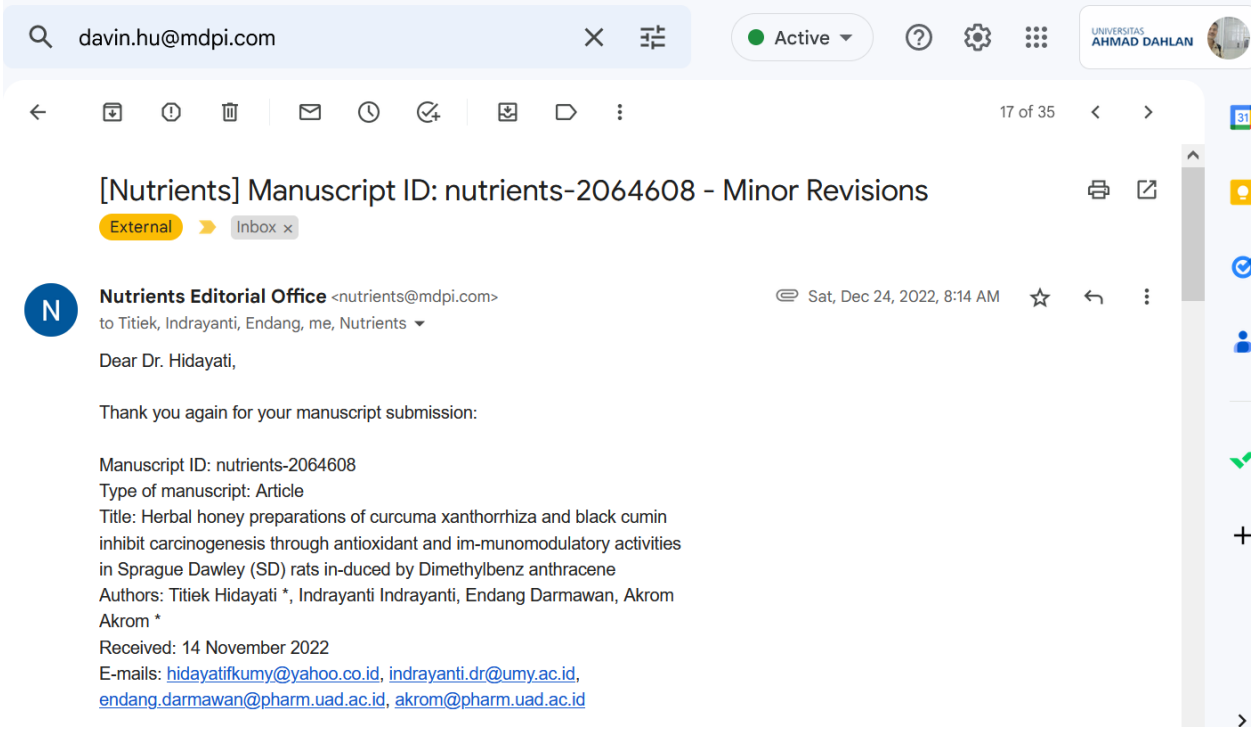
Nutrients Editorial Office <nutrients@mdpi.com>
to Titiek, Indrayanti, Endang, me, Nutrients

Thu, Dec 22, 2022, 8:13 AM

Dear Dr. Hidayati,

Thank you very much for providing the revised version of your paper:

Manuscript ID: nutrients-2064608
Type of manuscript: Article
Title: Herbal honey preparations of curcuma xanthorrhiza and black cumin inhibit carcinogenesis through antioxidant and im-munomodulatory activities in Sprague Dawley (SD) rats in-duced by Dimethylbenz anthracene
Authors: Titiek Hidayati *, Indrayanti Indrayanti, Endang Darmawan, Akrom Akrom *
Received: 14 November 2022
E-mails: hidayatifkumy@yahoo.co.id, indrayanti.dr@umy.ac.id, endang.darmawan@pharm.uad.ac.id, akrom@pharm.uad.ac.id



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17 of 35

[Nutrients] Manuscript ID: nutrients-2064608 - Minor Revisions

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Nutrients Editorial Office <nutrients@mdpi.com>
to Titiek, Indrayanti, Endang, me, Nutrients

Sat, Dec 24, 2022, 8:14 AM

Dear Dr. Hidayati,

Thank you again for your manuscript submission:

Manuscript ID: nutrients-2064608
Type of manuscript: Article
Title: Herbal honey preparations of curcuma xanthorrhiza and black cumin inhibit carcinogenesis through antioxidant and im-munomodulatory activities in Sprague Dawley (SD) rats in-duced by Dimethylbenz anthracene
Authors: Titiek Hidayati *, Indrayanti Indrayanti, Endang Darmawan, Akrom Akrom *
Received: 14 November 2022
E-mails: hidayatifkumy@yahoo.co.id, indrayanti.dr@umy.ac.id, endang.darmawan@pharm.uad.ac.id, akrom@pharm.uad.ac.id

Your manuscript has been reviewed by experts in the field. Please find your manuscript with the referee reports at this link:
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(I) Please revise your manuscript according to the referees' comments and upload the revised file within 2 days.
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(IV) Any revisions made to the manuscript should be marked up using the *highlight* function if you are using MS Word/LaTeX, such that changes can be easily viewed by the editors and reviewers.
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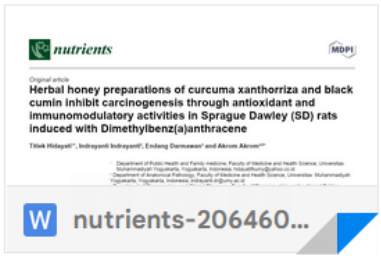

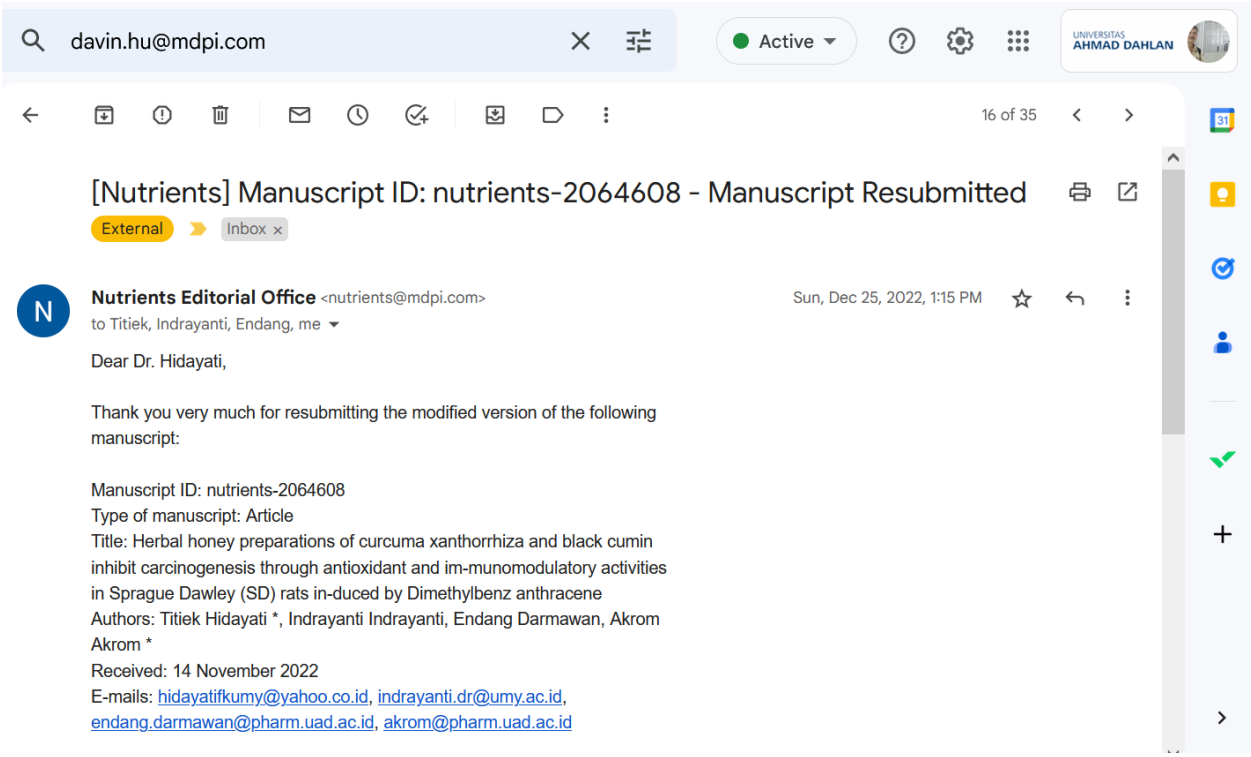

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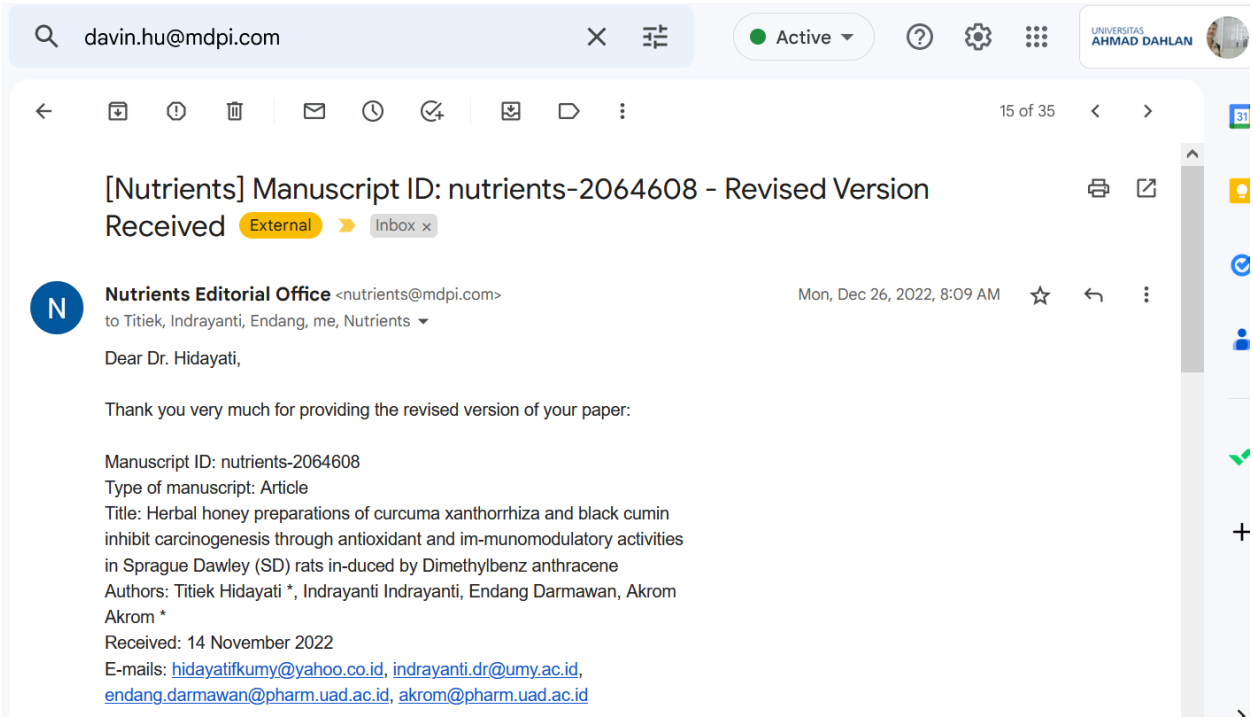

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3.One Giant Leap from Mouse to Man: The Microbiota–Gut–Brain Axis in Mood Disorders and Translational Challenges Moving towards Human Clinical Trials (http://www.mdpi.com/2072-6643/14/3/568)

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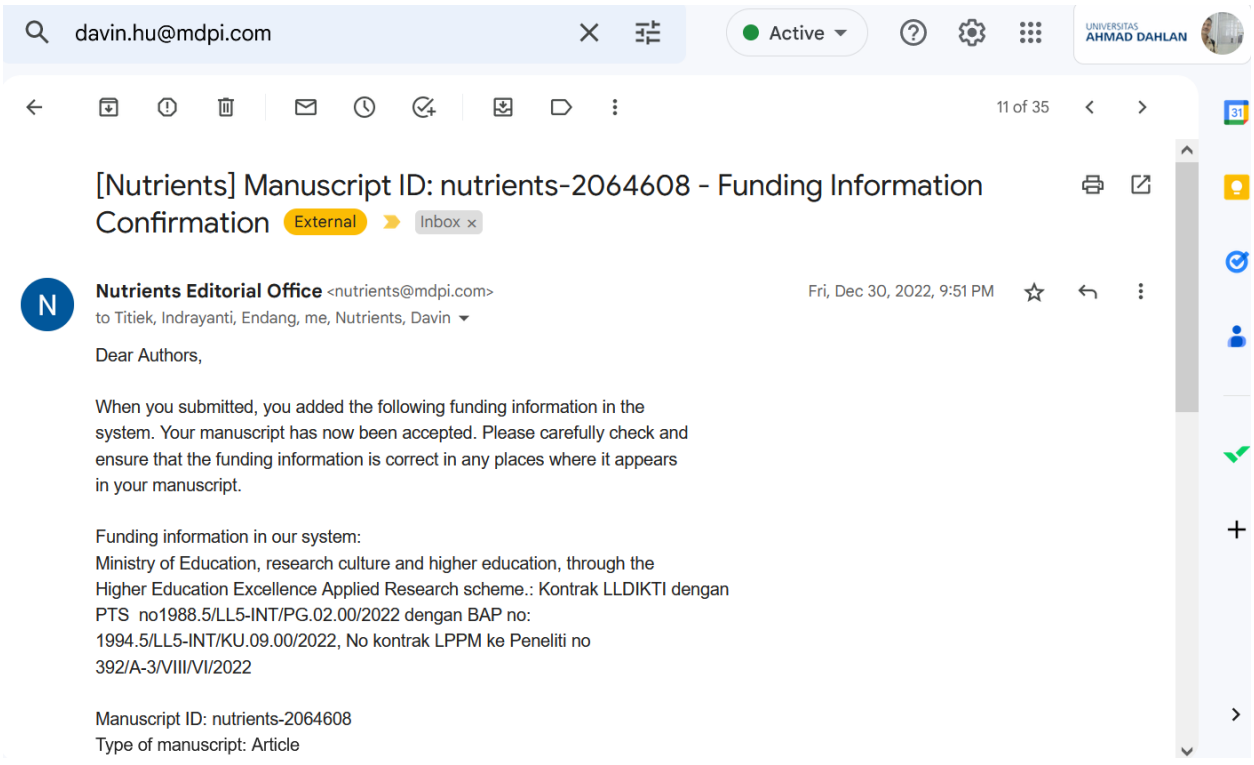

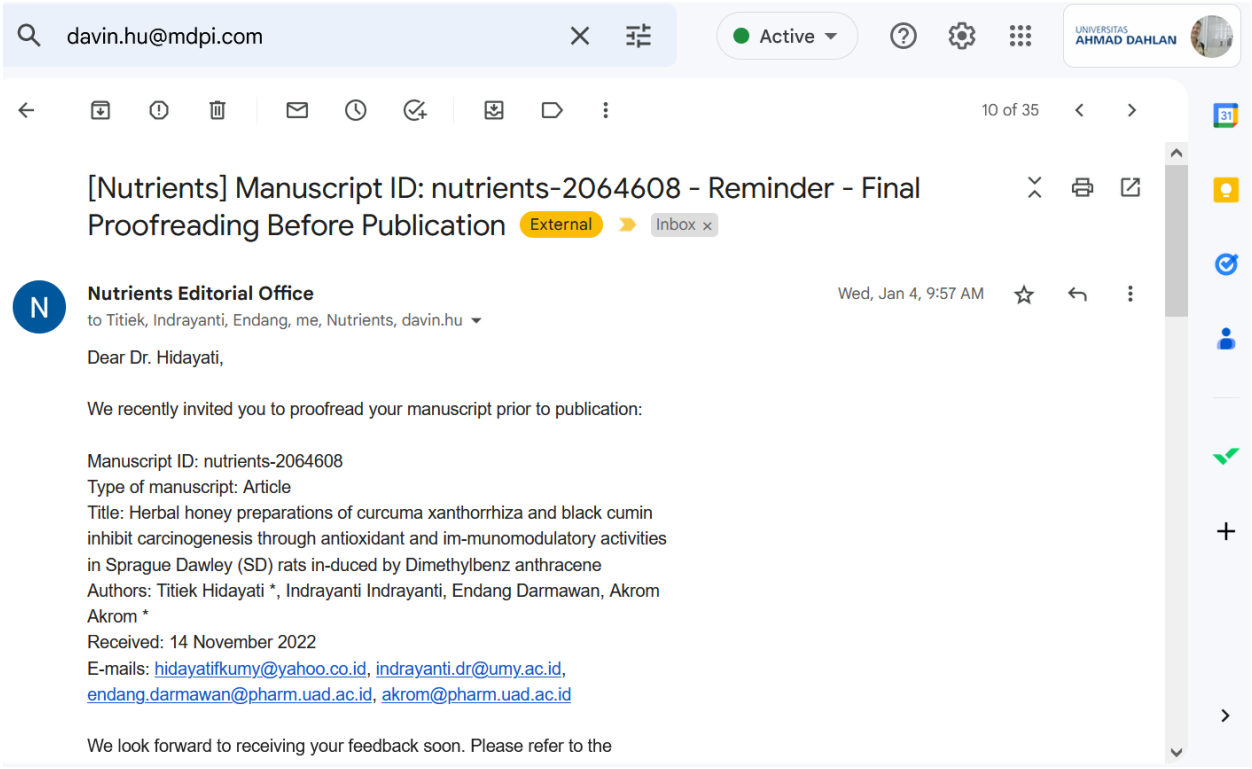

davin.hu@mdpi.com

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10 of 35

[Nutrients] Manuscript ID: nutrients-2064608 - Reminder - Final Proofreading Before Publication External Inbox ×

Nutrients Editorial Office
to Titiek, Indrayanti, Endang, me, Nutrients, davin.hu

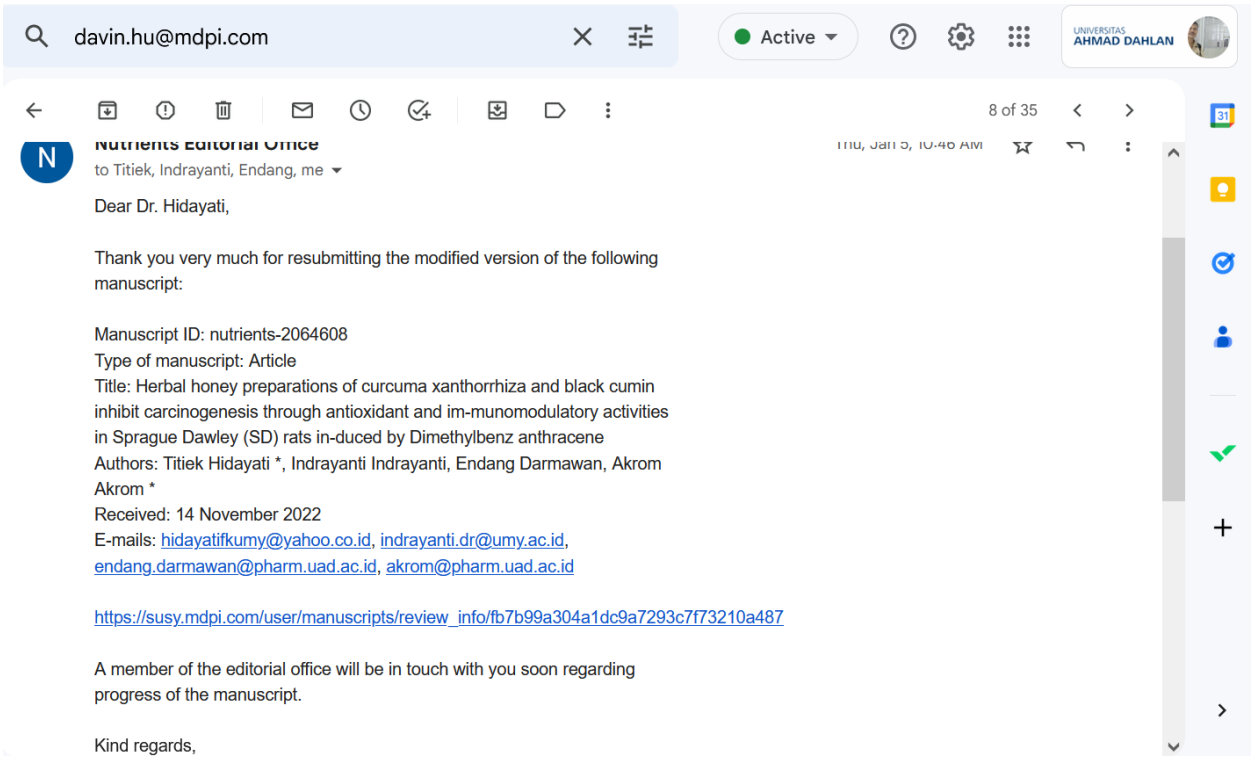
Wed, Jan 4, 9:57 AM

Dear Dr. Hidayati,

We recently invited you to proofread your manuscript prior to publication:

Manuscript ID: nutrients-2064608
Type of manuscript: Article
Title: Herbal honey preparations of curcuma xanthorrhiza and black cumin inhibit carcinogenesis through antioxidant and im-munomodulatory activities in Sprague Dawley (SD) rats in-duced by Dimethylbenz anthracene
Authors: Titiek Hidayati *, Indrayanti Indrayanti, Endang Darmawan, Akrom Akrom *
Received: 14 November 2022
E-mails: hidayatifkumy@yahoo.co.id, indrayanti.dr@umy.ac.id, endang.darmawan@pharm.uad.ac.id, akrom@pharm.uad.ac.id

We look forward to receiving your feedback soon. Please refer to the



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8 of 35

Nutrients Editorial Office
to Titiek, Indrayanti, Endang, me

Thu, Jan 5, 10:46 AM

Dear Dr. Hidayati,

Thank you very much for resubmitting the modified version of the following manuscript:

Manuscript ID: nutrients-2064608
Type of manuscript: Article
Title: Herbal honey preparations of curcuma xanthorrhiza and black cumin inhibit carcinogenesis through antioxidant and im-munomodulatory activities in Sprague Dawley (SD) rats in-duced by Dimethylbenz anthracene
Authors: Titiek Hidayati *, Indrayanti Indrayanti, Endang Darmawan, Akrom Akrom *
Received: 14 November 2022
E-mails: hidayatifkumy@yahoo.co.id, indrayanti.dr@umy.ac.id, endang.darmawan@pharm.uad.ac.id, akrom@pharm.uad.ac.id

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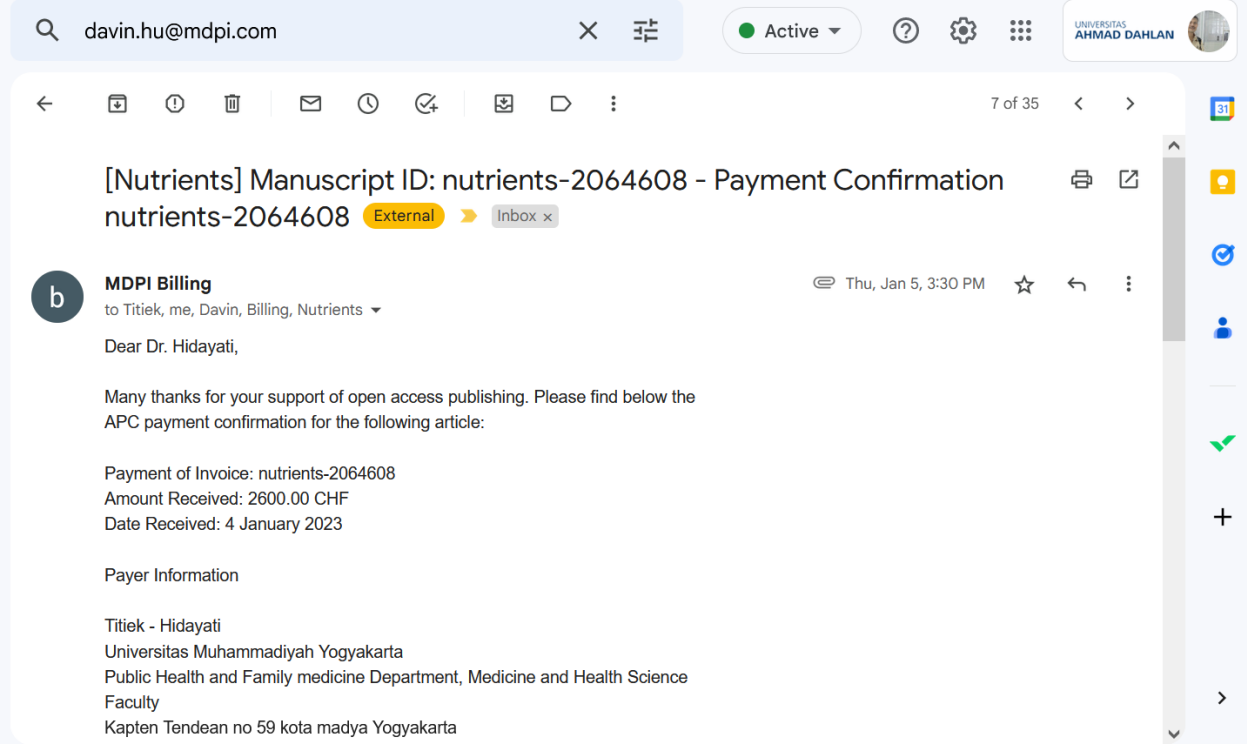

Titiek - Hidayati
Universitas Muhammadiyah Yogyakarta
Public Health and Family medicine Department, Medicine and Health Science Faculty
Kapten Tendean no 59 kota madya Yogyakarta
55252 Yogyakarta
Indonesia

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Please feel free to contact us if you have any questions.

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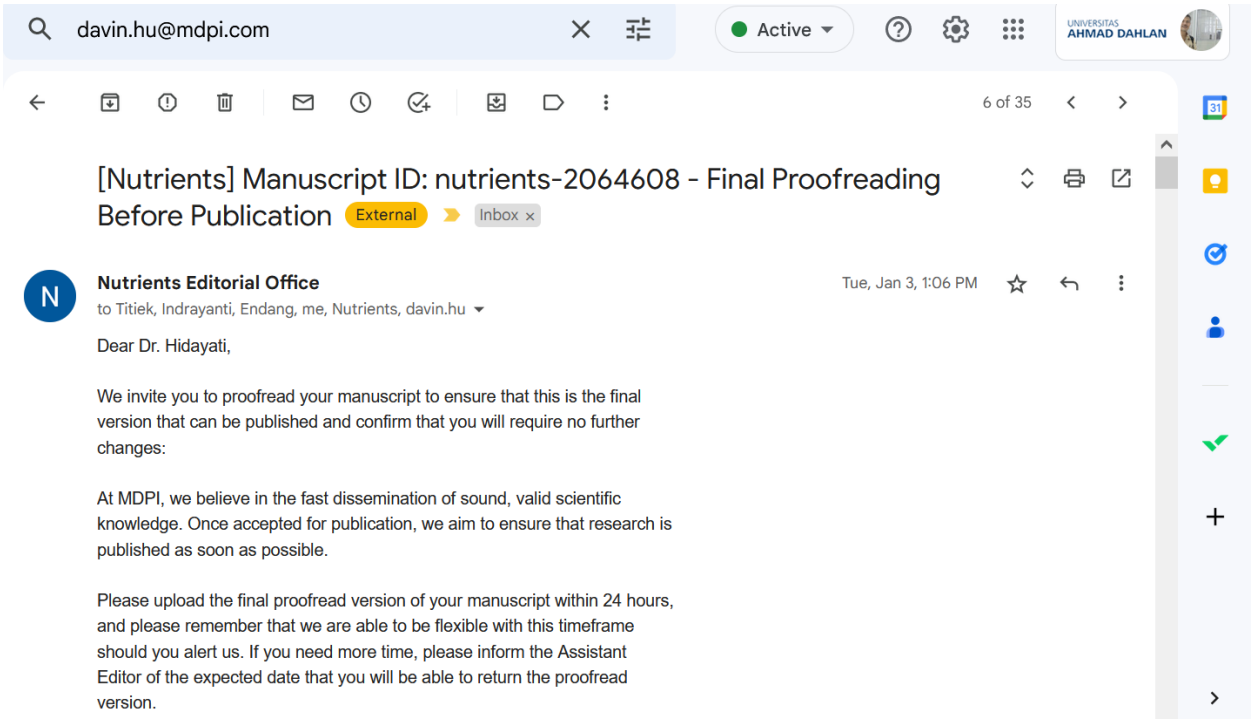

Manuscript ID: nutrients-2064608
Type of manuscript: Article
Title: Herbal honey preparations of curcuma xanthorrhiza and black cumin inhibit carcinogenesis through antioxidant and im-munomodulatory activities in Sprague Dawley (SD) rats in-duced by Dimethylbenz anthracene
Authors: Titiek Hidayati *, Indrayanti Indrayanti, Endang Darmawan, Akrom Akrom *
Received: 14 November 2022
E-mails: hidayatifkumy@yahoo.co.id, indrayanti.dr@umy.ac.id, endang.darmawan@pharm.uad.ac.id, akrom@pharm.uad.ac.id

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This manuscript includes supplementary materials, which you can find at the second link, above. Please note that citations and references in Supplementary files are permitted provided that they also appear in the reference list of the main text. Please ensure that you proofread your supplementary materials and upload them together with the manuscript.

We look forward to hearing from you soon.

Kind regards,
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Assistant Editor
E-Mail: davin.hu@mdpi.com

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---

davin hu Jan 5, 2023, 11:21 AM
to Titiek, Nutrients, Indrayanti, Endang, me

Dear Dr. Hidayati,

Thank you for your proofreading.

After checking, two problems still need to be solved.

(1)Please remove all the red wave lines in figure 1
(2)References [18] and [19] are repeated, please check. If they are same, and no new alternative references need to be added, please just confirm, then we will help you to remove the duplicate one. And rearrange the references.

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Thank you for your cooperation.

Kind regards,
Mr. Davin Hu
Assistant Editor

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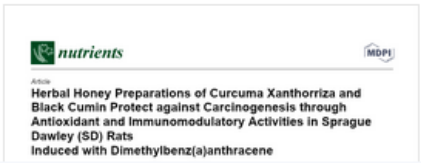
nutrients

Herbal Honey Preparations of Curcuma Xanthorriza and Black Cumin Protect against Carcinogenesis through Antioxidant and Immunomodulatory Activities in Sprague Dawley (SD) Rats Induced with Dimethylbenz(a)anthracene

Mr Akrom yk <akrom@pharm.uad.ac.id> Jan 5, 2023, 2:17 PM
to davin

Dear Editor

We have revised the draft according to the Editor's suggestion. Drafts attached.

Thank you

Best regard

Akrom

•••

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davin hu
to Titiek, Nutrients, Indrayanti, Endang, me

Jan 6, 2023, 8:27 AM

Dear Dr. Hidayati,

Thank you for your proofreading.

After checking, two problems still need to be solved as soon as possible.

(1)Please remove all the red wave lines in figure 1.
(2)References [18] and [19] are repeated, please check. If they are same, and no new alternative references need to be added, please just confirm, then we will help you to remove the duplicate one. And rearrange the references.

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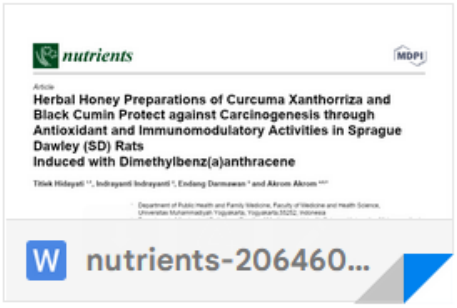

nutrients@mdpi.com
to Titiek, davin, Indrayanti, Endang, me

Jan 6, 2023, 3:30 PM

Dear Dr. Hidayati,

Thank you for your proofreading.

After checking, two problems still need to be solved as soon as possible.

(1)Please remove all the red wave lines in figure 1.
(2)References [18] and [19] are repeated, please check. If they are same, and no new alternative references need to be added, please just confirm, then we will help you to remove the duplicate one. And rearrange the references.

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Thank you for your cooperation.

Kind regards,
Amy Xie
Managing Editor

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Mr Akrom yk <akrom@pharm.uad.ac.id>
to davin, Titiek, Indrayanti, Endang

Jar

Thank you, I will do that.

Mr Akrom yk <akrom@pharm.uad.ac.id> Jan 6, 2023, 4:56 PM

to davin

Dear Editor

Herewith we attach the revised draft results.
Thank you.
Best Regard
Hidayati and Akrom

•••

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[Nutrients] Manuscript ID: nutrients-2064608; doi: 10.3390/nu15020371. Paper has been published.

External Inbox ×

nutrients@mdpi.com Wed, Jan 11, 7:36 PM

to hidayatifkumy, indrayanti.dr, endang.darmawan, me, billing, website, nutrients, marija.aleksic, davin.hu

Dear Authors,

We are pleased to inform you that your article "Herbal Honey Preparations of Curcuma Xanthorriza and Black Cumin Protect against Carcinogenesis through Antioxidant and Immunomodulatory Activities in Sprague Dawley (SD) Rats Induced with Dimethylbenz(a)anthracene" has been published in Nutrients and is available online:

Website: https://www.mdpi.com/2072-6643/15/2/371
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davin hu Thu, Jan 12, 9:06 AM

to hidayatifkumy, indrayanti.dr, endang.darmawan, me, nutrients

Dear Authors,

Congratulations that your paper "Herbal Honey Preparations of Curcuma Xanthorriza and Black Cumin Protect against Carcinogenesis through Antioxidant and Immunomodulatory Activities in Sprague Dawley (SD) Rats Induced with Dimethylbenz(a)anthracene" has been published in Nutrients. Please carefully check your paper (Website: https://www.mdpi.com/2072-6643/15/2/371). If there are incorrect contents, please inform us within 24 hours. Please kindly understand in this stage we do not accept any changes in linguistic issues.

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[Nutrients] Manuscript ID: nutrients-2064608 - Accepted for Publication

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Nutrients Editorial Office <nutrients@mdpi.com> Fri, Dec 30, 2022, 9:50 PM

to Titiek, Indrayanti, Endang, me, Nutrients, Davin

Dear Dr. Hidayati,

Congratulations on the acceptance of your manuscript, and thank you for submitting your work to Nutrients:

Manuscript ID: nutrients-2064608
Type of manuscript: Article
Title: Herbal honey preparations of curcuma xanthorrhiza and black cumin inhibit carcinogenesis through antioxidant and im-munomodulatory activities in Sprague Dawley (SD) rats in-duced by Dimethylbenz anthracene
Authors: Titiek Hidayati *, Indrayanti Indrayanti, Endang Darmawan, Akrom Akrom *
Received: 14 November 2022
E-mails: hidayatifkumy@yahoo.co.id, indrayanti.dr@umy.ac.id, endang.darmawan@pharm.uad.ac.id, akrom@pharm.uad.ac.id

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