Acknowledgement

8 pesan

Managing Editor <info@foodandnutritionjournal.org> 30 Maret 2018 17.40
Kepada: mutiara_nugraheni@uny.ac.id

Dear Author,

Greetings

Thank you for the submission.

Please send us the names, addresses, research areas and e-mail addresses of five potential reviewers.

Attached is the similarity report of your paper.

Please reframe the highlighted sentences using new words and make sure all the sources mentioned have been properly cited.

The similarity should not be more than 15%.

Best Regards

Sheen Shaikh
Content Specialist
Current Research in Nutrition and Food Science
www.foodandnutritionjournal.org

Managing Editor <info@foodandnutritionjournal.org> 30 Maret 2018 17.41
Kepada: mutiara_nugraheni@uny.ac.id

[Cutipan teks disembunyikan]

Potential_of_Ethanol_Extracts_of_Coleus_tuberosus_.pdf
298KB

Mutiara Nugraheni <mutiara_nugraheni@uny.ac.id> 1 April 2018 09.04
Kepada: Managing Editor <info@foodandnutritionjournal.org>

Dear editor,

Thank you for your response to our article. We send 5 potential reviewers, report plagiarism and articles that I have revised it and I check the level of plagiarism.
Sincerely yours,

Mutiara Nugraheni
[Kutipan leks disembunyikan]

5 lampiran

- Five potential reviewers.docx
  13K
- Plagiarism article reports with Plagiarism detector.pdf
  163K
- Plagiarism report Article Submit Mutiara Nugraheni without references.doc
  949K
- Revise Article Submit, Mutiara Nugraheni, with references.doc
  1194K
- Revise Article Submit, Mutiara Nugraheni, without references.doc
  1147K

Managing Editor <info@foodandnutritionjournal.org> 2 April 2018 13.43
Kepada: mutiara_nugraheni <mutiara_nugraheni@uny.ac.id>

Dear Mutiara Nugraheni

Greetings

Thank you for the revised article.

Please send the manuscript with references.

As per our new guidelines after the initial check the article goes through multiple step review process. Firstly the article is send to two reviewers simultaneously for quality analysis. In the next step it is forwarded to the Editor in Chief for final approval. Once approved at both levels then only it is accepted for publication.

This whole process takes time(5-6 weeks). We will update you accordingly.

Your patience will be deeply appreciated.

Best Regards

Sheen Shaikh
Content Specialist
Current Research in Nutrition and Food Science
www.foodandnutritionjournal.org
From: mutiara_nugraheni <mutiara_nugraheni@uny.ac.id>
Sent: Sunday, April 1, 2018 7:34 AM
To: Managing Editor
Subject: Re: Acknowledgement

[Cutipan teks disembunyikan]

--------------------------------------

Untuk mendukung "Gerakan UNY Hijau", disarankan tidak mencetak email ini dan lampirannya.

(To support the "Green UNY movement", it is recommended not to print the contents of this email and its attachments)

Universitas Negeri Yogyakarta
www.uny.ac.id

--------------------------------------

-mutiara_nugraheni <mutiara_nugraheni@uny.ac.id> 2 April 2018 14.03
Kepada: Managing Editor <info@foodandnutritionjournal.org>

Dear editor,

I send the manuscript with references. Thank you for your attention.

Sincerely yours,

Mutiara Nugraheni

[Cutipan teks disembunyikan]

[Manuscript_with references, Mutiara Nugraheni.doc
1194K]

-mutiara_nugraheni <mutiara_nugraheni@uny.ac.id> 28 Mei 2018 21.05
Kepada: Managing Editor <info@foodandnutritionjournal.org>

Dear editor,

My name mutiara nugraheni from Indonesia. I would like to ask about the process of our manuscript. Thank you for your attention.

Sincerely yours,

Mutiara Nugraheni

[Cutipan teks disembunyikan]
Managing Editor <info@foodandnutritionjournal.org>
Kepada: - mutiara_nugraheni <mutiara_nugraheni@uny.ac.id>

Dear Mutiara Nugraheni,

Greetings

Your article is under review. As we get two reviews done for each article, the review process generally takes time.

We will send you the reports soon.

Best Regards

Sheen Shaikh
Content Specialist
Current Research in Nutrition and Food Science
www.foodandnutritionjournal.org

From: - mutiara_nugraheni [mailto:mutiara_nugraheni@uny.ac.id]
Sent: Monday, May 28, 2018 7:36 PM

[Cutipan teks disembunyikan]

[Cutipan teks disembunyikan]

- mutiara_nugraheni <mutiara_nugraheni@uny.ac.id>
Kepada: Managing Editor <info@foodandnutritionjournal.org>

Thank you for the information.

Sincerely yours,

Mutiara
[Cutipan teks disembunyikan]
Dear Author,

Greetings

As per our guidelines we follow two step review process for refining the content quality of our journal. Attached are the two reports of your article.

We request you to go through the reports and send us a final highlighted revised file including corrections suggested by reviewers. Also send us two individual response forms (1 & 2) addressing both the reviewers separately along with one revised manuscript.

Best Regards

Ms. Sheen Shaikh
Content Specialist
Current Research in Nutrition and Food Science
www.foodandnutritionjournal.org

6 liripan

- R1.docx
  53K
- Comments 1.doc
  1198K
- R2.doc
  66K
- Comments 2.doc
  27K
- Response Form 1.docx
  50K
- Response Form 2.docx
  50K

16 Juli 2018 00:12
Current Research in Nutrition and Food Science – Review Form

- Title: “Potential of Ethanol Extracts of Coleus tuberosus Flesh and Peel as Natural Antioxidant and Breast Cancer Prevention Agents”
- Conflict of Interest: None
- Does the paper meet a high standard of scientific quality and credibility? Yes
- Is the paper readable and appropriately presented? No
- English language level: is the English language comprehensive and flawless? No, article need to be edited by professional English language editors.
- Does the paper contain appropriate referencing and any recognizable plagiarism? There is no recognizable plagiarism.
- Level of Interest: Please indicate how interesting you found the manuscript. The article is quite interesting, however has significant methodical errors.
- Is the paper compliant with the aims and scope of the journal it is submitted to? Yes
- Does the paper meet ethical requirements? Yes
- Other Comments?

The current study was designed to investigate the anti-cancerous potential of Ethanol Extracts of Coleus tuberosus Flesh and Peel on breast cancer cell line. Though, the aim of the study is clearly defined, some of the experiments need to be repeated. The article require major English editing.

Comments per section of manuscript

<table>
<thead>
<tr>
<th>Section</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abstract</td>
<td>Well written except few grammatical errors.</td>
</tr>
<tr>
<td>Introduction</td>
<td>Not focused, it need to be improved.</td>
</tr>
<tr>
<td>Methodology</td>
<td>Methods needs to be rewritten (please see attached word file and comments). There are many extra sentences which do not have any relevance and can be deleted. The language needs significant improvement. The authors performed MTT assay after one hour incubation with test compounds. MTT assay is usually performed for 24-72 hours.</td>
</tr>
</tbody>
</table>
How can author add 1 mL MTT solution in a well of 96 well plate? How volume of 96 well plate is around 300 microliter. How can author record MTT assay absorbance at 1 nm? Correct it. The study did not include any control (Cells treated with PMA alone)

<table>
<thead>
<tr>
<th>Results and Discussion</th>
<th>The language needs significant improvement. Professional English editing services may be consulted.</th>
</tr>
</thead>
<tbody>
<tr>
<td>References ( Appropriateness)</td>
<td>More references need to be added in Introduction.</td>
</tr>
</tbody>
</table>

**Rating (1 to 5) 1: Excellent, 5: Poor**

<table>
<thead>
<tr>
<th>Originality</th>
<th>2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Depth of research</td>
<td>3</td>
</tr>
<tr>
<td>Technical quality</td>
<td>4</td>
</tr>
</tbody>
</table>

**Recommendation:**
The manuscript may be after major revision or rejected unconditionally.
Title: Potential of Ethanol Extracts of *Coleus tuberosus* Flesh and Peel as Natural Antioxidant and Breast Cancer Prevention Agents

Conflict of Interest: NO

Please check the review policy at [www.foodandnutritionjournal.org/submission/review-guidelines/](http://www.foodandnutritionjournal.org/submission/review-guidelines/)

Does the paper meet a high standard of scientific quality and credibility? ☐ Yes ☐ No

Is the paper readable and appropriately presented? ☐ Yes ☐ No

English language level: is the English language comprehensive and flawless? ☐ Yes ☐ No

Are there any grammatical or spelling mistakes? ☐ Yes ☐ No

Are full forms for abbreviations stated at the 1st mention of the abbreviation? ☐ Yes ☐ No

Are appropriate legends provided with tables/figures? ☐ Yes ☐ No

Does the paper contain appropriate referencing and any recognizable plagiarism? ☐ Yes ☐ No

Level of Interest: Please indicate how interesting you found the manuscript.

“Overall this is an interesting study with potential explanations for the use of Ethanol Extracts of *Coleus tuberosus* Flesh and Peel as Natural Antioxidant for the treatment of breast cancer. Collectively, this study conducted meticulously and appears to be well designed and the results support the conclusion. The experiments are clearly presented, and the results appear solid and their analyses are reasonable. Simultaneously, I have some minor suggestions need to be addressed by authors. Could you please provide latest information in the introduction section regarding the role of other natural products in breast cancer (See Muhammad et al, Bitter melon extract inhibits breast cancer growth in preclinical model by inducing autophagic cell death. Oncotarget 2017 Aug 3;8(39):66226-66236. The manuscript looks very fascinating if authors make a schematic diagram of their findings in the above reference, which would be helpful for the readers”
In this study authors describe the significance of ethanol extract of Coleus tuberous flesh and peel as a natural antioxidant in the prevention of breast cancer model. Specifically, they try to link the relationship between ethanol extract of Coleus tuberous flesh and peel and their implication in the apoptosis, inhibition of cell proliferation, cell cycle arrest. In this study authors implies various types of techniques such as how to prepared the ethanoic extract of flesh and peel, antioxidant activity using ROS measurement, MTT assay, cell cycle analysis and apoptotic assay etc.

Overall this is an interesting study with potential explanations for the use flesh and peel as a natural antioxidant in the prevention of breast cancer. Collectively, this study conducted
meticulously and appears to be well designed and the results support the conclusion. The experiments are clearly presented, and the results appears solid and their analyses are reasonable. Simultaneously, I have some minor suggestions need to be addressed by authors.

Comments:

1. Could you please provide latest information in the introduction section regarding the role of other natural products in breast cancer (See Muhammad et al, Bitter melon extract inhibits breast cancer growth in preclinical model by inducing autophagic cell death. Oncotarget 2017 Aug 3;8(39):66226-66236. The manuscript looks very fascinating if authors make a schematic diagram of their findings in the above reference, which would be helpful for the readers”

2. In the abstract celullat should be cellular.

3. Please check the wave length in MTT assay.

4. Please describe the conc. of PMA used for induction of ROS.

5. It would be very nice, if authors could short the length of manuscript.

Rating (1 to 5) 1: Excellent, 5: Poor

<table>
<thead>
<tr>
<th>Originality</th>
<th>3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Depth of research</td>
<td>3</td>
</tr>
<tr>
<td>Technical quality</td>
<td>2</td>
</tr>
</tbody>
</table>

Recommendation:

☐ Reject unconditionally

☐ Reject in current form, but allow resubmission after revision as per my accompanying comments

☐ Accept conditionally, subject to minor revision, according to my accompanying comments
☐ Accept unconditionally
Kepada: Managing Editor <info@foodandnutritionjournal.org>

Dear editor,

Thank you for the opportunity to revised our manuscript that submitted. At this time, I submitted the revised article and respond to the comments of reviewers. I hope the revision in acceptable. But, there is something to be fixed, I am ready to immediately revised. Thank you for your attention.

Sincerely yours,

Mutiara Nugraheni

3 lampiran

- Revised article.doc 1205K
- Response Form 1.doc 64K
- Response Form 2.doc 62K

Managing Editor <info@foodandnutritionjournal.org> Kepada: - mutiara_nugraheni <mutiara_nugraheni@uny.ac.id>

Dear Dr. Mutiara Nugraheni,

Greetings

Thank you for the revised manuscript.

We will get back to you after the final comments from the Editor.

Best Regards

Ms. Sheen Shaikh
Content Specialist
Current Research in Nutrition and Food Science
www.foodandnutritionjournal.org
Comments after re-evaluation from the reviewer

3 pesan

Managing Editor <info@foodandnutritionjournal.org> 7 Agustus 2018 13.29
Kepada: mutiara_nugraheni@uny.ac.id

Dear Author,

As per the Editorial Suggestion, your manuscript has been sent to reviewer for re-evaluation and he has requested few more corrections to be incorporated in the final file as under:

"The manuscript still has many grammatical errors which need to be corrected. Manuscript can be accepted after language editing."

Thus please incorporate the above changes, so that we can proceed with the publication process.

Best Regards

Ms. Sheen Shaikh
Content Specialist
Current Research in Nutrition and Food Science
www.foodandnutritionjournal.org

-mutiara_nugraheni <mutiara_nugraheni@uny.ac.id> 7 Agustus 2018 16.01
Kepada: Managing Editor <info@foodandnutritionjournal.org>

Thank you. We will revised the manuscript for language editing.
[Kutipan teks disembunyikan]

-mutiara_nugraheni <mutiara_nugraheni@uny.ac.id> 13 Agustus 2018 22.49
Kepada: Managing Editor <info@foodandnutritionjournal.org>

Dear editor,

I have been doing the language editing in our manuscript. We hope this revision as expected of editors.
Thank you for your attention.

Sincerely yours.

Mutiara Nugraheni
[Kutipan teks disembunyikan]
2 lampiran

- Editorial Certificate_Mutiara.pdf
  316K
- Mutiara_13082018.doc
  1209K
Acceptance cum Bill
5 pesan

Managing Editor <info@foodandnutritionjournal.org> 14 Agustus 2018 14.12
Kepada: mutiara_nugraheni <mutiara_nugraheni@uny.ac.id>

Dear Dr. Mutiara,

Greetings!

Thanks for the revised paper.

Attached is the Acceptance cum Bill for your Paper.

We would like to inform you that your paper was reviewed by our editorial Committee and is accepted for the issue of August 2018.

You are requested to kindly send the publication charges at the earliest as our coming issue is closing on 20th August.

Kindly transfer the charges on the following account details below:

Account Name: Enviro research publishers
Bank: State Bank of India
Branch: Bhopal main branch , T.T. Nagar Bhopal- 462 001
Account No.:32679196050
Swift no. SBININBB268

Best Regards

Ms. Sheen Shaikh
Content Specialist
Current Research in Nutrition and Food Science
www.foodandnutritionjournal.org
From: mutiara_nugraheni [mailto:mutiara_nugraheni@uny.ac.id]
Sent: Monday, August 13, 2018 9:20 PM
To: Managing Editor
Subject: Re: Comments after re-evaluation from the reviewer

Dear editor,

I have been doing the language editing in our manuscript. We hope this revision as expected of editors. Thank you for your attention

Sincerely yours,

Mutiara Nugraheni

2018-08-07 16:01 GMT+07:00 - mutiara_nugraheni <mutiara_nugraheni@uny.ac.id>:
Thank you. We will revised the manuscript for language editing.

Selasa, 07 Agustus 2018, Managing Editor <info@foodandnutritionjournal.org> menulis:
Dear Author,

As per the Editorial Suggestion, your manuscript has been sent to reviewer for re-evaluation and he has requested few more corrections to be incorporated in the final file as under:

"The manuscript still has many grammatical errors which need to be corrected. Manuscript can be accepted after language editing."

Thus please incorporate the above changes, so that we can proceed with the publication process.

Best Regards

Ms. Sheen Shaikh
Content Specialist
Current Research in Nutrition and Food Science
www.foodandnutritionjournal.org
Dear editor,
I sent the publication charge. Thank you

Sincerely yours,

Mutiara Nugraheni

[Cut off text]

Managing Editor <info@foodandnutritionjournal.org> 16 Agustus 2018 13.47
Kepada: mutiara_nugraheni@uny.ac.id

Dear Mutiara Nugraheni,

Greetings

Thank you. We will check and update you.

Best Regards
Email Universitas Negeri Yogyakarta - Acceptance cum Bill

Ms. Sheen Shaikh
Content Specialist
Current Research in Nutrition and Food Science
www.foodandnutritionjournal.org

From: mutiara_nugraheni@uny.ac.id
Sent: Wednesday, August 15, 2018 12:40 PM
To: Managing Editor
Subject: Re: Acceptance cum Bill

Dear Ms. Sheen Shaikh,

Please send me information related to articles that have been accepted and have sent proof of publication charge, based on information to be published to volume 6 (2), please give me progress report the process of our article. If we can get the pdf file for the purposes of evidence in our University. Thank you for your attention.

Sincerely yours,

Mutiara Nugraheni

Managing Editor <info@foodandnutritionjournal.org>
Kepada: mutiara_nugraheni@uny.ac.id

Dear Mutiara,

Your article has already been forwarded for publication.

Once the paper is online we will notify you.

Best Regards

Ms. Sheen Shaikh
Content Specialist
Current Research in Nutrition and Food Science

www.foodandnutritionjournal.org

From: mutiara_nugraheni [mailto:mutiara_nugraheni@uny.ac.id]
Sent: Thursday, August 23, 2018 5:25 AM

[Kutipan teks disembunyikan]

[Kutipan teks disembunyikan]
Request for Final PDF check
1 pesan

Managing Editor <info@foodandnutritionjournal.org> 24 Agustus 2018 18.04
Kepada: mutiara_nugraheni <mutiara_nugraheni@uny.ac.id>

Dear Dr. Mutiara,

Greetings!

Hope this mail finds you well.

Your article has been forwarded for online publication.

Meanwhile please go through the attached PDF proof and let us know if any corrections needed.

Our August issue is closing next week.

Best Regards

Ms. Sheen Shaikh
Content Specialist
Current Research in Nutrition and Food Science
www.foodandnutritionjournal.org

Mutiara Nugraheni.pdf 617K
Request for Final PDF check (URGENT)

11 pesan

Managing Editor <info@foodandnutritionjournal.org> 27 Agustus 2018 01.53

Kepada: mutiara_nugrahenci@uny.ac.id

Dear Dr. Mutiara,

Greetings!

Hope this mail finds you well.

Your article has been forwarded for online publication.

Meanwhile please go through the attached PDF proof and let us know if any corrections needed.

Our august issue is closing this week.

Best Regards

Ms. Sheen Shaikh

Content Specialist

Current Research in Nutrition and Food Science

www.foodandnutritionjournal.org

- Mutiara Nugrahenci.pdf

617K

Mutiara Nugrahenci <mutiara_nugrahenci@uny.ac.id> 27 Agustus 2018 06.15

Kepada: Managing Editor <info@foodandnutritionjournal.org>

Dear Ms. Sheen Shaikh

I submit a correction to a pdf article that was sent to me. Thank you

Sincerely yours,

Mutiara Nugrahenci

[ Kutipan teks disembunyikan]

- Current research in Nutrition and Food science 2018 Revised.pdf

617K

Managing Editor <info@foodandnutritionjournal.org> 27 Agustus 2018 11.14

Kepada: mutiara_nugrahenci@uny.ac.id
From: mutiara_nugraheni [mailto:mutiara_nugraheni@uny.ac.id]
Sent: Monday, August 27, 2018 4:45 AM
To: Managing Editor
Subject: Re: Request for Final PDF check (URGENT)

[Kutipan teks disembunyikan]

_________________________________________________________________________________

Untuk mendukung "Gerakan UNY Hijau", disarankan tidak mencetak email ini dan lampirannya.

(To support the "Green UNY movement", it is recommended not to print the contents of this email and its attachments)

Universitas Negeri Yogyakarta
www.uny.ac.id

_________________________________________________________________________________

Current research in Nutrition and Food science 2018 Revised.pdf
617K

Managing Editor <info@foodandnutritionjournal.org>
Kepada: mutiara_nugraheni <mutiara_nugraheni@uny.ac.id>

28 Agustus 2018 12.17

Dear Mutiara Nugraheni,

The corrections have been successfully incorporated. Kindly approve.

Best Regards

Ms. Sheen Shaikh
Content Specialist
Current Research in Nutrition and Food Science
www.foodandnutritionjournal.org

From: mutiara_nugraheni [mailto:mutiara_nugraheni@uny.ac.id]
Dear Ms. Sheen Shaikh

I'm sorry, there is one correction again in final check. In scientific name of Coleus tuberosus. I send in attach file. And when this correction complete, I approve for publish on line.

Thank you

Best regards,

Mutiara Nugraheni

---

Untuk mendukung "Gerakan UNY Hijau", disarankan tidak mencetak email ini dan lampirannya.

(To support the "Green UNY movement", it is recommended not to print the contents of this email and its attachments)

Universitas Negeri Yogyakarta
www.uny.ac.id

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Mutiara Nugraheni.pdf
622K

- mutiara_nugraheni <mutiara_nugraheni@uny.ac.id> 28 Agustus 2018 15.32
Kepada: Managing Editor <info@foodandnutritionjournal.org>

Dear Ms. Sheen Shaikh

I'm sorry, there is one correction again in final check. In scientific name of Coleus tuberosus. I send in attach file. And when this correction complete, I approve for publish on line.

Thank you

Best regards,

Mutiara Nugraheni

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Mutiara Nugraheni Revised.pdf
614K

- mutiara_nugraheni <mutiara_nugraheni@uny.ac.id> 28 Agustus 2018 15.55
Kepada: Managing Editor <info@foodandnutritionjournal.org>

Dear Ms. Sheen Shaikh

I'm sorry, there is two correction again in final check. In scientific name of Coleus tuberosus and extracts (with s). I send in attach file. And when this correction complete, I approve for publish on line.

Thank you

Best regards,
Mutiara Nugraheni

Managing Editor <info@foodandnutritionjournal.org>
Kepada: mutiara_nugraheni <mutiara_nugraheni@uny.ac.id>
28 Agustus 2018 16.16

From: mutiara_nugraheni [mailto:mutiara_nugraheni@uny.ac.id]
Sent: Tuesday, August 28, 2018 2:25 PM

Dear editor,

The correction in my article: extract ... please replace with extracts (add s) and Coleus tuberosus in Table 2...IC50...please write in italic. Thank you

Best Regards

Mutiara Nugraheni

Managing Editor <info@foodandnutritionjournal.org>
Kepada: mutiara_nugraheni <mutiara_nugraheni@uny.ac.id>
31 Agustus 2018 16.57

Dear Dr. Mutiara Nugraheni,

Greetigs!

As per your request the correction has been done.

Thank you for publishing with us.

Best Regards
Ms. Sheen Shaikh  
Content Specialist  
Current Research in Nutrition and Food Science  
www.foodandnutritionjournal.org

From: mutiara_nugraheni [mailto:mutiara_nugraheni@uny.ac.id]  
Sent: Friday, August 31, 2018 5:11 AM  
To: Managing Editor  
Subject: Re: FW: Request for Final PDF check (URGENT)

Dear editor,

I proved the revised article. Thanl you for your attention

Best regards,

Mutiara nugraheni.

Managing Editor <info@foodandnutritionjournal.org>  
Kepada: mutiara_nugraheni <mutiara_nugraheni@uny.ac.id>  
31 Agustus 2018 17.58

Dear Mutiara nugraheni,

Greetings!

Thank you for the cooperation.

Best Regards

Ms. Sheen Shaikh  
Content Specialist  
Current Research in Nutrition and Food Science
Our April Issue (6-2) is Online

1 pesan

Managing Editor <info@foodandnutritionjournal.org> 1 September 2018 15.34
Kepada: Managing Editor <info@foodandnutritionjournal.org>

Dear Author,

Greetings!

The editorial office would like to inform you that the current issue Volume-6 Issue-2 is online now. Articles of the August Issue (6-2) are now available to read and download.

You can view it on: http://www.foodandnutritionjournal.org/current-issue/

We would like to thank you for considering Current Research in Nutrition and Food Science Journal for publication.

Kindly like our social media pages, the links are given below:

FACEBOOK: https://www.facebook.com/foodandnutritionjournal/
LINKEDIN: https://www.linkedin.com/company/current-research-in-nutrition-and-food-science-journal/?trk=biz-companies-cym
TWITTER: https://twitter.com/crnfsjournal

We welcome more submissions from you in future.

Best Regards

Ms. Sheen Shaikh
Content Specialist
Current Research in Nutrition and Food Science
www.foodandnutritionjournal.org
Our August Issue(6-2) is Online

1 pesan

Managing Editor <info@foodandnutritionjournal.org> 3 September 2018 11.16
Kepada: Managing Editor <info@foodandnutritionjournal.org>

Dear Author,

Greetings!

The editorial office would like to inform you that the current issue Volume-6 Issue-2 is online now. Articles of the August Issue(6-2) are now available to read and download.

You can view it on: http://www.foodandnutritionjournal.org/current-issue/

We would like to thank you for considering Current Research in Nutrition and Food Science Journal for publication.

Kindly like our social media pages, the links are given below:

FACEBOOK: https://www.facebook.com/foodandnutritionjournal/
LINKEDIN: https://www.linkedin.com/company/current-research-in-nutrition-and-food-science-journal/?trk=biz-companies-cym
TWITTER: https://twitter.com/crnfsjournal

We welcome more submissions from you in future.

Best Regards

Ms. Sheen Shaikh
Content Specialist
Current Research in Nutrition and Food Science
www.foodandnutritionjournal.org
ABSTRACT

This study evaluates the effects of ethanol extracts of *Coleus tuberosus* flesh and peel on T47D cancer cells. Antioxidant potential was evaluated with cellular antioxidant activity experiment, and anti-proliferation activity was evaluated by MTT assay (3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide). The effects on cell cycle were evaluated with a flow cytometry, while induction ofed apoptosis was evaluated based on morphological changes by staining with acridine orange and ethidium bromide. The peel extract had higher cellular antioxidant activity than the flesh extract. The IC<sub>50</sub> of cellular antioxidant activity of the flesh and peel extracts were 287.13±10.35 µg/ml and 217.86±12.96 µg/ml, respectively. The peel extract also had higher anti-proliferative activity. The IC<sub>50</sub> of anti-proliferative activity were 887.05±5.03 µg/ml (flesh extract) and 548.18±4.52 µg/ml (peel extract). The peel and flesh extracts can cause cell cycle arrest in the S phase and G2-M phase. The extracts induce apoptosis within T47D cancer cells, which show an orange color. Therefore, these extracts could be used as potential sources of natural antioxidants and breast cancer prevention agents.

Keywords: antioxidant, anti-proliferation, cell cycle, apoptosis, ethanol extract of *Coleus tuberosus*

Introduction

Cancer is caused by a disruption of the normal regulation of cell growth control. In Indonesia, breast cancer is the second most common cause of cancer-related death after cervix cancer and has a prevalence of 0.01%. The number of breast cancer patients is increasing and the occurrence has reached 1 in every 10,000 women. Data from the Ministry of Health revealed that 1.4 in every 1,000 women had breast cancer in 2013 (a total of around 347,000 people). In Indonesia, 21.5 deaths among every 100,000 people are caused by breast cancer. Alarmingly, 70 percent of new breast cancer patients only visit health centers at an advanced stage.
Cancer cases are linked to diet and lifestyle\textsuperscript{2}. Several studies have investigated the anti-proliferation effects of some phytochemicals derived from fruits and vegetables. Studies are examining cancer prevention by utilizing foods that contain bioactive compounds such as antioxidants\textsuperscript{3,4}. More than 1,000 different phytochemicals have been identified as having potential effects against various cancers. These phytochemicals offer considerable advantages because they are safe and may target multiple cell-signaling pathways\textsuperscript{5}. Related to the risk of the occurrence of breast cancer are high enough in Indonesia, T47D cancer cells often used as research models to know the ability of anti-proliferation of bioactive compounds contained in fruits, vegetables, cereals, legume or tubers.

\textit{Coleus tuberosus} is a tubers as source of carbohydrate, and some research suggests that it has potential antioxidant and anti-proliferation effects. Bioactive compounds identified in the \textit{Coleus tuberosus} include phenols, flavonoids, oleanolic acid, ursolic acid, maslinic acid, and phytosterol, which have antioxidant, and antiproliferation, apoptosis effects\textsuperscript{6,7,8}. Further research is necessary on the antioxidant activity and cancer prevention activity. Thus, this study examines the potential of \textit{Coleus tuberosus} extract as natural antioxidant and breast cancer prevention agents using T47D cancer cells.

\textbf{Materials and methods}

\textbf{Chemicals}

This study investigated the effects of ethanol extracts of \textit{Coleus tuberosus} flesh and peel on T47D cancer cell line. MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) (Sigma Aldrich), RPMI (Sigma Aldrich), phosphate
buffered saline (PBS) (Gibco), DMSO (Sigma Aldrich), and acridine orange - ethidium bromide (Bio Rad) were used.

**Preparation of ethanol extracts of Coleus tuberosus flesh and peel**

The peels of Coleus tuberosus were separated with peeler from the flesh with a thickness of 1 mm. The flesh and peel were dried with a cabinet dryer for twenty four hours and then crushed and extracted using maceration with 95% ethanol for 7 days. The extracts were stored in a frozen condition.

**Evaluation of cellular antioxidant activity of ethanol extracts of Coleus tuberosus flesh and peel in T47D cancer cells**

The cellular antioxidant activity was evaluated by referring to Wolfe and Liu\(^9\) and Liu and Finley\(^10\).

T47D cells grown on a 96-well microplate containing RPMI supplemented with fetal bovine serum 10% (v/v), penicillin 100 U, and streptomycin 100 mg/ml at 37°C under 5% CO\(_2\). After 24 hours of growth, growth medium was removed, and the wells were washed with sterile PBS. The cells were pretreated with ethanol extracts of Coleus tuberosus flesh or peel in concentrations of 100, 200, 400, and 800 µg/ml for 20 min in triplicate. After 20 min of pretreatment, growth media containing 25 µM DCFH-DA and PMA (100 ng/ml in DMSO) was added and incubated for 30 minutes. The fluorescence of the cells was measured using a flow cytometer (FACS Calibur BD) at a wavelength of 535 nm. The cellular antioxidant activity was determined by calculating the percentage decrease in the intensity of the reactive oxygen species (ROS) fluorescence:

\[
\text{Decreased ROS of percentage} = \left( \frac{\text{Fit}_0 - \text{Fit}_1}{\text{Fit}_0 - \text{Fit}_2} \right) \times 100\%
\]
where $F_{i_0}$ is the oxidative stress control, $F_{i_1}$ is the cells with bioactive compound treatment, and $F_{i_2}$ is that of the control without oxidative stress$^{11}$.

**Evaluation of anti-proliferation of T47D cancer cell lines**

The anti-proliferation effects were evaluated by referring to Hogan et al.$^{12}$. Cells were placed on a 96-well plate at $1.5 \times 10^4$ cells/ml with RPMI plus 10% (v/v) fetal bovine serum, penicillin 100 U and streptomycin (100 mg/ml, 37°C, 5% CO$_2$). These conditions were kept for a one-hour period. Next, cancer cells were treated for about one hour in the experiment media with ethanol extract of *Coleus tuberosus* peel or flesh extracts at concentrations of 62.5-2,000 µg/ml. Cell viability was determined by MTT assay. After a one-hour incubation, the media were treated and eliminated at the end of the incubation period and then washed with HBSS. The cells were incubated with 1 mL of MTT reagent solution (0.5 mg/ml in RPMI) added to each of the wells for one hour.

The mixtures were left for one night. The absorbance was recorded at 1 nm with a multipliable plate reader. The absorbance data required for cell viability are expressed as a percentage of the control (the number of living cells in control cells) during the experiment. After each treatment, the MTT assay was expressed as:

$\text{Cell viability (\%) = \left(\text{absorbance of treatment group: absorbance without treatment}\right) \times 100\%}$

**Cell cycle arrest**

A total of $10^6$ cell/well were distributed in 6-well plates and incubated at 37°C for cell adaptation. T47D cells incubated with ethanol extract of *Coleus tuberosus* peel or flesh extracts with concentrations of 7.8125, 15.625, 31.25, 62.5 and 125 µg/ml for
24 hours. After 24 hours, cells incubated taken and washed twice using ice-cold PBS. The cells were fixed and permeabilized with 70% ice-cold ethanol at 4°C for one hour. Then T47D cells washed with PBS and resuspended in a solution containing propidium iodide stain (50 µl/ml) and RNase A (250 µg/ml). Cell suspensions were incubated for 30 min at room temperature, followed by fluorescence-activated cell sorting (FACS; cater-plus flow cytometry; Becton Dickinson co., Germany) using 10,000 cells per group\textsuperscript{13}. The percentage of cells in the G0-G1, S, and G2/M phases were analyzed using Modfit LT Cell Cycle 3.0 analysis software (Becton Dickinson).

**Induction of apoptosis**

The morphological changes due to apoptosis induction after treatment with the extracts were evaluated using acridine orange staining and ethidium bromide. T47D cells cultured on the cover slip on 10\textsuperscript{5} cells/well. The medium was replaced with medium containing samples ethanol extract of *Coleus tuberosus* flesh or peel extracts (62.5 µg/ml). The cells were then incubated for 24 hours at 37°C in 5% CO\textsubscript{2}. The medium was removed and solution of acridine orange and ethidium bromide (100 µg/ml of acridine orange (Bio-Rad) in PBS and 100 µg/ml of ethidium bromide (Bio-Rad) in PBS for 5 minutes) was added. The cover slips were mounted on a glass object and then observed under a fluorescence microscope (Zeiss MC 80)\textsuperscript{14}.

**Statistical analysis**

Each experiment was periformed three times and data were expressed as mean ± SD The testing was performed by ANOVA of the antioxidant activity, anti-proliferation
effects and cell cycle arrest. If the obtained results difference significantly, then continued with Duncan’s Multiple Range Test (DMRT)

Results and discussion

Cellular antioxidant activity of ethanol extracts of *Coleus tuberosus* flesh and peel on T47D

Treatment of T47D cancer cells with ethanol extracts of *Coleus tuberosus* flesh (EECF) or ethanol extract of *Coleus tuberosus* peel (EECP) inhibited PMA-induced ROS generation in dose-dependent manner. The percentage decrease of ROS in cancer cells incubated with peel extract T47D was higher than that with cells incubated with ethanol extract of *Coleus tuberosus* flesh or peel (Fig. 1).

The percentage decreases of ROS in the T47D cancer cells incubated with ethanol extract of *Coleus tuberosus* peel extract extract at concentration of 100, 200, 400, and 800 µg/ml were 31.30 ± 3.27, 46.93 ± 9.78, 64.96 ± 7.23, and 77.09 ± 1.78, respectively (Fig.1). The percentage decreases of ROS in the T47D cells incubated with ethanol extract of *Coleus tuberosus* flesh at concentrations of 100, 200, 400, and 800 µg/ml were 22 ± 7.93, 38.70 ± 5.49, 55.96 ± 2.04, and 62.08 ± 1.54, respectively.
The percentage decrease in reactive oxygen species (ROS) with the treatment of ethanol extract of *Coleus tuberosus* flesh and peel on T47D cells induced with phorbol myristate acetate

Note: Different notations suggest a significant difference (p < 0.05).

The cellular antioxidant activity of peel extract on T47D cells was higher than that of flesh extract, which is shown with the IC$_{50}$ values. The IC$_{50}$ of the flesh extract was 287.13 ± 10.35 µg/ml, whereas that of the peel extract was 217.86 ± 12.96 µg/ml (Table 1).

Table 1. IC$_{50}$ cellular antioxidant activities of ethanol extracts of *Coleus tuberosus* flesh and peel on T47D cancer cells

<table>
<thead>
<tr>
<th>Bioactive compounds</th>
<th>Cellular antioxidant activity IC$_{50}$ (µg/ml)</th>
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<tbody>
<tr>
<td>Ethanol extract of <em>Coleus tuberosus</em> flesh</td>
<td>287.13±10.35</td>
</tr>
<tr>
<td>Ethanol extract of <em>Coleus tuberosus</em> peel</td>
<td>217.86±12.96</td>
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</table>

The current study investigated the antioxidant activity and anti-proliferation activity of ethanol extracts of *Coleus tuberosus* flesh and peel on T47D cancer cell lines. This results show that the extracts have the ability to decrease ROS in T47D cells induced by PMA. The ability decrease are associated with content of bioactive compounds in *Coleus tuberosus*, such as phenols, flavonoids, oleanolic acid, ursolic acid, maslinic acid, and phytosterol$^{6,15,16}$. Some research suggests that incubation with extracts containing bioactive compounds may lower the ROS in cancer cells induced by free radicals and improve the antioxidant defense system$^{17-21}$. Therefore, treatment with bioactive compounds could prevent DCFH oxidation and decrease the formation of fluorescent DCF$^{11,22,23}$. 

Fig 1. The percentage decrease in reactive oxygen species (ROS) with the treatment of ethanol extract of *Coleus tuberosus* flesh and peel on T47D cells induced with phorbol myristate acetate.
The antioxidative ability of the flesh and peel extracts is from the interaction between bioactive compounds in the extract, which have a synergistic effect. This supports research conducted by Náthia-Neves et al.\textsuperscript{24} and Li et al.\textsuperscript{25}, who showed the difference in antioxidant ability related to the difference in content of bioactive compounds in extracts. A high amount of bioactive compounds tends to result in high ability to neutralize free radicals.

The mechanism of bioactive compounds could have the ability to maintain the fluidity of cell membranes by capturing ROS, so cellular level communication can occur normally, including the entire signals associated with activation of antioxidant enzymes (Nrf-2-ACRE)\textsuperscript{26}. Increased expression of Nrf-2-ARE that helps increasing the antioxidant defense system (GPx, SOD, glutathione, CAT, vitamin C, vitamin E, and beta-carotene). An increase in the antioxidant defense system of cells can increase the ability to neutralize singlet oxygen ($^1$O$_2$), hydrogen peroxide (H$_2$O$_2$), superoxide anion radicals (O$_2^-$), and hydroxyl radicals (OH') due to the induction of PMA. An increase in glutathione, vitamin C, vitamin E, and beta-carotene in the cell can increase the capture of free radicals that are present in the cell and reduce the amount of free radicals in T47D cells.

**The effects of ethanol extract of *Coleus tuberosus* flesh and peel extracts on the proliferation of T47D cancer cells**

The anti-proliferative activity of flesh and peel extract on T47D cancer cells was characterized by MTT assay. Cells were treated by applying different extract concentrations of 62.5, 125, 250, 500, 1000, and 2000 µg/ml and incubated for 24 hours (Fig. 2).
Fig. 2. The percentage of viability cells produced by treatments with an ethanol extract of *Coleus tuberosus* flesh (EECF) and peel (EECP) at different concentrations after a 24-hour incubation. Data are presented as the mean of three independent experiments. Means followed by different letters is significant difference (p < 0.05).

Fig. 2 indicates that the incubation of T47D cancer cells with flesh extract and peel extract can decrease the percentage of viable cells compared to the control in a dose-dependent manner. The anti-proliferative activity of peel extract and flesh extract is expressed as the inhibition concentration (IC$_{50}$). A lower IC$_{50}$ value indicates a higher anti-proliferative effect of a sample. Table 2 shows that the IC$_{50}$ levels after a 24-hour incubation period are 887.05 ± 5.03 µg/ml (peel extract) and 548.18 ± 4.52 µg/ml (flesh extract). The peel extract seems to have a higher anti-proliferative effect than the flesh extract at all concentrations, which corresponds to its lower IC$_{50}$.

Table 2. IC$_{50}$ anti-proliferation activities of ethanol extracts of *Coleus tuberosus* flesh and peel on T47D cancer cells

<table>
<thead>
<tr>
<th>Bioactive compounds</th>
<th>IC$_{50}$ (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ethanol extract of <em>Coleus tuberosus</em> flesh</td>
<td>887.05±5.03</td>
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</table>
The peel extract and flesh extract may cause a significant growth inhibition of T47D in a dose-dependent manner. In particular, the peel extract showed higher anti-proliferative activities than flesh extract towards T47D cancer cells. These results are in line with the previous research by Nugraheni et al.\(^7\), who proved that peel extract has a greater anti-proliferation activity than flesh extract. The inhibition ability for the proliferation of cancer cells is supported by the presence of various bioactive compounds in the flesh and peel, such as carotenoids, vitamins, and other polyphenol phytochemicals. The peels also contain more bioactive compounds than the flesh\(^27\).

The anti-proliferation activities of the flesh extract and peel extract on T47D cancer cells may have been caused by interactions between bioactive compounds in the extracts. These bioactive compounds include ursolic acid, oleanolic acid\(^6\), phenol and flavonoids compounds\(^15\), maslinic acid, and phytosterols, such as beta-sitosterol, stigmasterol, and campesterol\(^8\). These provide a synergy effect in determining the anti-proliferation ability. Prior research also suggests that maslinic acid, phytosterols, and phenolic compounds may have anti-proliferative activity in some cancer cells \(^28\)-\(^32\). In particular, ursolic acid and oleanolic acid are known as being capable of inhibiting the proliferation of cancer cells\(^7,33,34\).

**Cell cycle arrest**

Evaluation using flow cytometer used to know the effect of ethanol extract of *Coleus tuberosus* flesh and peel on cell cycle arrest (125, 62.5, 31.25, 15.625, and 7.8125 µg/ml). Cell cycle arrest occurs in the S and G2/M phases (Fig. 3 and Fig. 4).
This indicates that treatment with flesh extract or peel extract causes changes in the cancer cell cycles.

Fig. 3. Effect of ethanol extract of Coleus tuberosus flesh on cell cycle arrest. T47D cells were treated with 0 (control), 7.8125, 15.625, 31.25, 62.5, and 125 µg/ml of peel extract for 24 h, after which the cells were stained with PI and analyzed for DNA content by flow cytometer. Means followed by different letters differ statistically (p < 0.05).
Fig. 4. Effect of ethanol extract of *Coleus tuberosus* peel extract on cell cycle arrest. T47D cells were treated with 0 (control), 7.8125, 15.625, 31.25, 62.5, and 125 µg/ml of peel extract for 24 h, after which the cells were stained with PI and analyzed for DNA content by flow cytomter. Means followed by different letters differ statistically (p < 0.05).

T47D cells incubated with ethanol extract of *Coleus tuberosus* flesh or peel showed cell cycle arrest in the S and G2/M phases, in a dose dependent manner. Some research suggests that bioactive compounds such as: flavonoids, polyphenols, ursolic acid and oleanolic acid are potent regulators which can cause the S-phase and G2-M cell cycle arrest\textsuperscript{35-39}. The ability of ethanol extract of flesh and peel of *Coleus tuberosus* induces cell cycle arrest could cause an inhibition of cell proliferation.

**Acridine orange staining-ethidium bromide on T47D cells**

The phenotypic characteristics of cells, which are separately treated with flesh extract and peel extract, are evaluated by conducting a microscopic inspection of the
overall morphology. The results show that either the flesh extract or peel extract performs a significant evidence of cells death even after the 24-hour incubation period. Staining with acridine orange and ethidium bromide on all treatment is used to find out the capabilities of extracts in inducing apoptosis on T47D cells. The treated cells indicate a nuclear condensation after the 24-hour treatment. The positive control cells show bright green nuclei (Fig. 5A), while the negative control cells produce an orange-red color with uniform intensity (Fig. 5B). The apoptotic cells appear to have an orange color (Fig. 5C).

<table>
<thead>
<tr>
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<th>Ethanol extract of Coleus tuberosus flesh</th>
<th>Ethanol extract of Coleus tuberosus peel</th>
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<tbody>
<tr>
<td>Negative control</td>
<td>Untreated cells (No apoptotic cells) (A)</td>
<td></td>
</tr>
<tr>
<td>Positive control</td>
<td>(apoptotic cells) (B)</td>
<td></td>
</tr>
<tr>
<td>Treatment:</td>
<td>Treatment with concentration of 62.5 µg/ml (C)</td>
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**Fig. 5.** A. Untreated cells, B. treated cells with doxorubicin, C. treatment with concentration of 62.5 µg/ml. Cellular and nuclear morphological changes of T47D
cancer cells due to exposure to various concentrations of *Coleus tuberosus* flesh or peel extract after a 24-hour incubation. Viable cells have a uniform colour and bright green with an organized structure. Early apoptotic cells have green nuclei, but the perinuclear chromatin condensation is visible as bright green fragment or patches (red arrow). Late apoptotic cells indicated have orange to red-colored nuclei with fragmented chromatin (white arrow).

Treatment with T47D cancer cells with ethanol extract of *Coleus tuberosus* flesh and peel induced apoptosis, which was characterized by changing shape, condensation, and DNA degradation of the cells (Figure 5). The combination of acridine orange and ethidium bromide can be used on both living and dead cells undergoing apoptosis. Green fluorescence occurs when bound to double-stranded DNA in living cells, and red fluorescence occurs when bound to single-stranded DNA, predominantly in dead cells. Cells that experience apoptosis early will experience fragmentation of DNA, which results in the green color of the nucleus. When apoptotic processes end, DNA fragmentation results in a red or orange, because cells lose the integrity of the membrane, so that the ethidium bromide can enter into the cell and intercalate with the DNA that has undergone fragmentation. Shikha Srivastava et al. indicated that the treatment of cancer cells by flavonoids led to the fragmentation and degradation of cellular DNA and resulting in apoptosis.

This research shows that ethanol extract of *Coleus tuberosus* flesh and peel have the ability as antioxidant activity on T47D cells, inhibiting the proliferation, induce cell cycle arrest and induce apoptosis. This result shows the potential of *Coleus tuberosus* as a source of natural antioxidants and has the ability to inhibit proliferation of T47D cancer cells.
Conclusion

Ethanol extracts of *Coleus tuberosus* flesh and peel can reduce reactive oxygen species in T47D cells induced with the radical generator PMA. The ability to reduce oxidative stress is large with the peel extract than the of flesh extract. Incubation of cells with the extracts can reduce proliferation, causing cell cycle arrest in the S and G2-M phases, and induced apoptosis.

Conflict of interest

The authors declare no conflict of interest in this work

Acknowledgement

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References

Ethanol Extracts of *Coleus tuberosus* Flesh and Peel as a Potential Source of Natural Antioxidant and Breast Cancer Prevention Agent

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Abstract
This study examines the effects of ethanol extracts of *Coleus tuberosus* flesh and peel on T47D cancer cells. Antioxidant potential was evaluated through cellular antioxidant activity experiment, and anti-proliferation activity was evaluated using MTT assay (3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide). The effects on cell cycle were evaluated with a flow cytometry, while induction of apoptosis was evaluated based on morphological changes by staining with acridine orange and ethidium bromide. The results indicate that the peel extract had higher cellular antioxidant activity than the flesh extract. The IC50 of cellular antioxidant activity of the flesh and peel extracts were 287.13±10.35 µg/ml and 217.86±12.96 µg/ml, respectively. The IC50 of anti-proliferative activity were 887.05±5.03 µg/ml (flesh extract) and 548.18±4.52 µg/ml (peel extract). The peel and flesh extracts can cause cell cycle arrest in the S phase and G2-M phase. The extracts induce apoptosis within T47D cancer cells, showing an orange color. Therefore, these extracts could be used as potential sources of natural antioxidants and breast cancer prevention agents.

Introduction
Breast cancer is the most common cancer and cause of cancer-related deaths among women, accounting for 1.67 million (25.2%) new cases and 521,907 (14.7%) deaths worldwide1. Breast cancer is a cancer that develops from the breast tissue. This is an invasive cancer that often appears in women. Some signs of breast cancer are a change of breast shape, dimpled skin, nipple discharge or red scaly patches of skin, and a lump in the breast2. Based on data of
the International Agency for Research on Cancer (IARC) in 2012, the incidence of cancer in women has reached 134 per 100,000 of the population. The highest incidence is breast cancer with 40 cases per 100,000 women followed by cervical cancer reaching 17 cases per 100,000. The estimate of the death toll in Indonesia Globocan for breast cancer is 16.6 deaths per 100,000 population.

Cancer cases are linked to diet and lifestyle. Although surgery and chemotherapy can lower the risks of breast cancer in some women who suffer the disease, the side effects cause them to limit the use. Currently, preventive efforts have become more important. Some natural products have an important role in the discovery and development of drugs for the treatment of various types of deadly diseases including cancer. Several studies have investigated the anti-proliferation effects of some phytochemicals derived from fruits and vegetables. Research examines cancer prevention by utilizing foods that contain bioactive compounds such as antioxidants. More than 1,000 different phytochemicals have been identified as having potential effects against various cancers. These phytochemicals offer considerable advantages because they are safe and may target multiple cell-signaling pathways. Related to the high occurrence of breast cancer in Indonesia, T47D cancer cells are often used as research models to identify the ability of anti-proliferation of bioactive compounds contained in fruits, vegetables, cereals, legume or tubers.

*Coleus tuberosus* is a source of carbohydrate. Some research suggests that it has potential antioxidant and anti-proliferation effects. Bioactive compounds identified in the *Coleus tuberosus* which include phenols, flavonoids, oleaonic acid, ursolic acid, maslinic acid, and phytosterol have antioxidant, and antiproliferation, apoptosis effects. Further research on the antioxidant activity and cancer prevention activity is still needed. Thus, this study examines the potential of *Coleus tuberosus* extract as natural antioxidant and breast cancer prevention agents using T47D cancer cells.

**Materials and Methods**

**Chemicals**

This study investigated the effects of ethanol extracts of *Coleus tuberosus* flesh and peel on T47D cancer cell line. MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) (Sigma Aldrich), RPMI (Sigma Aldrich), phosphate buffered saline (PBS) (Gibco), DMSO (Sigma Aldrich), and acridine orange - ethidium bromide (Bio Rad) were used.

**Preparation of Ethanol Extracts of Coleus Tuberosus Flesh and Peel**

The peels of *Coleus tuberosus* were separated with peeler from the flesh with a thickness of 1 mm. The flesh and peel were dried in a cabinet dryer for twenty four hours and then crushed and extracted using maceration with 95% ethanol for 7 days. The extracts were stored in a frozen condition.

**Evaluation of Cellular Antioxidant Activity of Ethanol Extracts of Coleus Tuberosus Flesh and Peel in T47D Cancer Cells**

The cellular antioxidant activity was evaluated by referring to Wolfe and Liu and Liu and Finley. T47D cells grown on a 96-well microplate containing RPMI were supplemented with fetal bovine serum 10% (v/v), penicillin 100 U, and streptomycin 100 mg/ml at 37°C under 5% CO₂. After 24 hours of growth, growth medium was removed, and the wells were washed with sterile PBS. The cells were pretreated with ethanol extracts of *Coleus tuberosus* flesh or peel in concentrations of 100, 200, 400, and 800 µg/ml for 20 mins in triplicate. After 20 mins of pretreatment, growth media containing 25 µM DCFH-DA and PMA (100 ng/ml in DMSO) were added and incubated for 30 minutes. The fluorescence of the cells was measured using a flow cytometer (FACS Calibur BD) at a wavelength of 535 nm. The cellular antioxidant activity was determined by calculating the percentage decrease in the intensity of the reactive oxygen species (ROS) fluorescence:

\[
\text{Decreased ROS of percentage} = \frac{F_{t_1} - F_{t_2}}{F_{t_2}} \times 100\%
\]

where \(F_{t_1}\) is the oxidative stress control, \(F_{t_2}\) is the cells with bioactive compound treatment, and \(F_{t_2}\) is that of the control without oxidative stress.

**Evaluation of Anti-Proliferation of T47D Cancer Cell Lines**

The anti-proliferation effects were evaluated by referring to Hogan et al. Cells were placed on a 96-well plate at 1.5x10⁴ cells/ml with RPMI plus 10% (v/v) fetal bovine serum, penicillin 100 U and...
streptomycin (100 mg/ml, 37°C, 5% CO\textsubscript{2}). These conditions were kept for a one-hour period. Next, cancer cells were treated for about one hour in the experiment media with ethanol extract of *Coleus tuberosus* peel or flesh extracts at concentrations of 62.5-2,000 µg/ml with the final volume of 100 µl. Cell viability was determined by MTT assay. After one-hour incubation, the media were treated and eliminated at the end of the incubation period and then washed with HBSS. The cells were incubated with 10 µL of MTT reagent solution (5 mg/ml in RPMI) added to each of the wells for 24 hours.

The mixtures were left for one night. The absorbance was recorded at 570 nm with a multipliable plate reader. The absorbance data required for cell viability are expressed as a percentage of the control (the number of living cells in control cells) during the experiment. After each treatment, the MTT assay was expressed as:

\[
\text{Cell viability} \, (\%) = \frac{(\text{Absorbance of treatment group})}{(\text{Absorbance without treatment})} \times 100\% 
\]

**Cell Cycle Arrest**

A total of 10\textsuperscript{5} cell/well were distributed in 6-well plates and incubated at 37°C for cell adaptation. T47D cells incubated with ethanol extract of *Coleus tuberosus* peel or flesh extracts at concentrations of 7.8125, 15.625, 31.25, 62.5 and 125 µg/ml for 24 hours. After 24 hours, cells incubated were taken and washed twice using ice-cold PBS. The cells were fixed and permeabilized with 70% ice-cold ethanol at 4°C for one hour. Then, T47D cells were washed with PBS and resuspended in a solution containing propidium iodide stain (50 µg/ml) and RNase A (250 µg/ml). Cell suspensions were incubated for 30 min at room temperature, followed by fluorescence-activated cell sorting (FACS; cater-plus flow cytometry; Becton Dickinson co., Germany) using 10,000 cells per group\textsuperscript{16}. The percentages of cells in the G0-G1, S, and G2/M phases were analyzed using Modfit LT Cell Cycle 3.0 analysis software (Becton Dickinson).

**Induction of Apoptosis**

The morphological changes due to apoptosis induction after treatment with the extracts were evaluated using acridine orange staining and ethidium bromide. T47D cells were cultured on the cover slip on 10\textsuperscript{5} cells/well. The medium was replaced with medium containing ethanol extract samples of *Coleus tuberosus* flesh or peel extracts (62.5 µg/ml). The cells were then incubated for 24 hours at 37°C in 5% CO\textsubscript{2}. The medium was removed and solution of acridine orange and ethidium bromide (100 µg/ml of acridine orange (Bio-Rad) in PBS and 100 µg/ml of ethidium bromide (Bio-Rad) in PBS for 5 minutes) was added. The cover slips were mounted on a glass object and then observed under a fluorescence microscope (Zeiss MC 80)\textsuperscript{17}.

**Statistical Analysis**

Each experiment was performed three times and data were expressed as mean ± SD. The testing was performed by two way anova of the antioxidant activity, anti-proliferation effects and cell cycle arrest. If the obtained results are different significantly, then it was followed with Duncan’s Multiple Range Test (DMRT).

**Results and Discussion**

**Cellular Antioxidant Activity of Ethanol Extracts of *Coleus Tuberosus* Flesh and Peel on T47D**

Treatment of T47D cancer cells with ethanol extracts of *Coleus tuberosus* flesh (EECF) or ethanol extract of *Coleus tuberosus* peel (EECP) inhibited PMA-induced ROS generation in dose-dependent manner. The percentage decrease of ROS in cancer cells incubated with peel extract T47D was higher than that of cells incubated with ethanol extract of *Coleus tuberosus* flesh or peel (Fig. 1).

The percentage decreases of ROS in the T47D cancer cells incubated with ethanol extract of *Coleus tuberosus* flesh at concentrations of 100, 200, 400, and 800 µg/ml were 22 ± 7.93, 38.70 ± 5.49, 55.96 ± 2.04, and 62.08 ± 1.54, respectively.

The cellular antioxidant activity of peel extract on T47D cells was higher than that of flesh extract, which is shown by the IC\textsubscript{50} values. The IC\textsubscript{50} of the flesh extract was of 287.13 ± 10.35 µg/ml, whereas that of the peel extract was 217.86 ± 12.96 µg/ml (Table 1).
The current study investigated the antioxidant activity and anti-proliferation activity of ethanol extracts of *Coleus tuberosus* flesh and peel on T47D cancer cell lines. The results show that the extracts have the ability to decrease ROS in T47D cells induced by PMA. The ability is associated with content of bioactive compounds in *Coleus tuberosus*, such as phenols, flavonoids, oleanolic acid, ursolic acid, maslinic acid, and phytosterol. Some research suggests that incubation with extracts containing bioactive compounds may lower the ROS in cancer cells induced by free radicals and improve the antioxidant defense system. Therefore, treatment with bioactive compounds could prevent DCFH oxidation and decrease the formation of fluorescent DCF.

The antioxidative ability of the flesh and peel extracts comes from the interaction between bioactive compounds in the extract, which have a synergistic effect. This supports the research of Náthia-Neves *et al.*, and Li *et al.*, indicating that the different antioxidant ability is related to the different content of bioactive compounds in extracts. A high amount of bioactive compounds tends to result in high ability to neutralize free radicals.

The mechanism of bioactive compounds could have the ability to maintain the fluidity of cell membranes by capturing ROS, so that cellular level communication can occur normally, including the entire signals associated with activation of antioxidant enzymes (Nrf-2-ACRE). Increased expression of Nrf-2-ARE can increase the antioxidant defense system (GPx, SOD, glutathione, CAT, vitamin C, vitamin E, and beta-carotene). Further, an increase in the antioxidant defense system of cells can increase the ability to neutralize singlet oxygen ($1O_2$), hydrogen peroxide ($H_2O_2$), superoxide anion radicals ($O_2^{•−}$), and hydroxyl radicals (OH•) due to the induction of PMA. An increase in glutathione, vitamin C, vitamin E, and beta-carotene in the cell can increase the capture of free radicals that are present in the cell and reduce the amount of free radicals in T47D cells.

### Table 1. IC$_{50}$ cellular antioxidant activities of ethanol extracts of *Coleus tuberosus* flesh and peel on T47D cancer cells

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Fig.1: The percentage decrease in reactive oxygen species (ROS) with the treatment of ethanol extract of *Coleus tuberosus* flesh and peel on T47D cells induced with phorbol myristate acetate

Note: Different notations suggest a significant difference ($p < 0.05$)
The Effects Of Ethanol Extract of *Coleus Tuberosus* Flesh and Peel Extracts on the Proliferation of T47D Cancer Cells

The anti-proliferative activity of flesh and peel extract on T47D cancer cells was characterized by MTT assay. Cells were treated by applying different extract concentrations of 62.5, 125, 250, 500, 1000, and 2000 µg/ml and incubating the cells for 24 hours (Fig. 2).

Fig. 2 indicates that the incubation of T47D cancer cells with flesh extract and peel extract can decrease the percentage of viable cells compared to the control in a dose-dependent manner. The anti-proliferative activity of peel extract and flesh extract is expressed as the inhibition concentration (IC$_{50}$). A lower IC$_{50}$ value indicates a higher anti-proliferative effect of a sample. Table 2 shows that the IC$_{50}$ levels after a 24-hour incubation period are 887.05 ± 5.03 µg/ml (peel extract) and 548.18 ± 4.52 µg/ml (flesh extract). The peel extract seems to have a higher anti-proliferative effect than the flesh extract at all concentrations, which corresponds to its lower IC$_{50}$.

![Fig. 2: The percentage of viability cells produced by treatments with an ethanol extract of *Coleus tuberosus* flesh (EECF) and peel (EECP) at different concentrations after a 24-hour incubation. Data are presented as the mean of three independent experiments. Means followed by different letters is significant difference (p < 0.05)](image)

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The peel extract and flesh extract may cause a significant growth inhibition of T47D in a dose-dependent manner. In particular, the peel extract showed higher anti-proliferative activities than the flesh extract towards T47D cancer cells. These results are in line with previous research by Nugraheni *et al.*, who proved that peel extract has a greater anti-proliferation activity than flesh extract. The inhibition ability for the proliferation of cancer cells is supported by the presence of various bioactive compounds in the flesh and peel, such as carotenoids, vitamins, and other polyphenol phytochemicals. The peels also contain more bioactive compounds than the flesh.
The anti-proliferation activities of the flesh extract and peel extract on T47D cancer cells may have been caused by the interactions between bioactive compounds in the extracts. These bioactive compounds include ursolic acid, oleanolic acid\textsuperscript{a}, phenol and flavonoids compounds\textsuperscript{b}, maslinic acid, and phytosterols, such as beta-sitosterol, stigmasterol, and campesterol\textsuperscript{c}. These provide a synergy effect in determining the anti-proliferation ability. Prior research also suggests that maslinic acid, phytosterols, and phenolic compounds may have anti-proliferative activity in some cancer cell\textsuperscript{31-35}. In particular, ursolic acid and oleanolic acid are known as being capable of inhibiting the proliferation of cancer cells\textsuperscript{10,36-37}.

**Cell Cycle Arrest**

Evaluation using flow cytometer used to identify the effect of ethanol extract of *Coleus tuberosus* flesh and peel on cell cycle arrest (125, 62.5, 31.25, 15.625, and 7.8125 µg/ml). Cell cycle arrest occurs in the S and G2/M phases (Fig. 3 and Fig. 4). This indicates that treatment with flesh extract or peel extract causes changes in the cancer cell cycles.

![Cell Cycle Arrest](image)

**Fig. 3**: Effect of ethanol extract of *Coleus tuberosus* flesh on cell cycle arrest. T47D cells were treated with 0 (control), 7.8125, 15.625, 31.25, 62.5, and 125 µg/ml of peel extract for 24 h, after which the cells were stained with PI and analyzed for DNA content by flow cytometer. Means followed by different letters differ statistically (p < 0.05).

T47D cells incubated with ethanol extract of *Coleus tuberosus* flesh or peel showed cell cycle arrest in the S and G2/M phases, in a dose dependent manner. Some research suggests that bioactive compounds such as flavonoids, polyphenols, ursolic acid and oleanolic acid are potent regulators which can cause the S-phase and G2-M cell cycle arrest\textsuperscript{38-42}. The ability of ethanol extract of flesh and peel of *Coleus tuberosus* induces the cell cycle arrest could cause an inhibition of cell proliferation.
Fig. 4: Effect of ethanol extract of *Coleus tuberosus* peel extract on cell cycle arrest. T47D cells were treated with 0 (control), 7.8125, 15.625, 31.25, 62.5, and 125 µg/ml of peel extract for 24 h, after which the cells were stained with PI and analyzed for DNA content by flow cytometer. Means followed by different letters differ statistically (p < 0.05).

Acridine Orange Staining-Ethidium Bromide on T47D Cells
The phenotypic characteristics of cells, which are separately treated with flesh extract and peel extract, were evaluated by conducting a microscopic inspection of the overall morphology. The results show that either the flesh extract or peel extract performs a significant evidence of cells death even after the 24-hour incubation period. Staining with acridine orange and ethidium bromide on all treatment was used to find out the capabilities of extracts in inducing apoptosis on T47D cells. The treated cells indicated a nuclear condensation after the 24-hour treatment. The positive control cells showed bright green nuclei (Fig. 5A), while the negative control cells produced an orange-red color with uniform intensity (Fig. 5B). The apoptotic cells had an orange color (Fig. 5C).

Cellular and nuclear morphological changes of T47D cancer cells due to exposure to various concentrations of *Coleus tuberosus* flesh or peel extract after a 24-hour incubation. Viable cells have a uniform colour, bright green with an organized structure. Early apoptotic cells have green nuclei, but the perinuclear chromatin condensation is visible as bright green fragment or patches (red arrow). Late apoptotic cells indicated have orange to red-colored nuclei with fragmented chromatin (white arrow).

Treatment with T47D cancer cells with ethanol extract of *Coleus tuberosus* flesh and peel induced apoptosis, which was characterized by changing shape, condensation, and DNA degradation of the cells (Figure 5). The combination of acridine orange and ethidium bromide can be used on both living and dead cells undergoing apoptosis. Green fluorescence occurs when bound to double-stranded DNA in living cells, and red fluorescence occurs when bound to single-stranded DNA, predominantly in dead cells. Cells that experience apoptosis early will experience fragmentation of DNA, which results in the green color of the nucleus. When apoptotic processes end, DNA fragmentation results in a red or orange, because cells lose the integrity of the membrane, so that the ethidium bromide can enter into the cell and intercalate with the DNA that has undergone fragmentation. Shikha Srivastava et al. indicated that the treatment of cancer cells by flavonoids led to the fragmentation and degradation of cellular DNA, resulting in apoptosis.
This research shows that ethanol extracts of *Coleus tuberosus* flesh and peel have the ability as antioxidant for T47D cells, inhibiting the proliferation, inducing cell cycle arrest and inducing apoptosis.

This results show the potential of *Coleus tuberosus* as a source of natural antioxidants which the ability to inhibit proliferation of T47D cancer cells.

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<td>Treatment: 62.5 µg/ml (C)</td>
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**Fig. 5:** A. Untreated cells, B. treated cells with doxorubicin, C. treatment with concentration of 62.5 µg/ml

**Conclusion**
Ethanol extracts of *Coleus tuberosus* flesh and peel can reduce reactive oxygen species in T47D cells induced with the radical generator PMA. The ability of the peel extract to reduce oxidative stress is better than that of the flesh extract. Incubation of cells with the extracts can reduce proliferation, causing cell cycle arrest in the S and G2-M phases, and induced apoptosis.

**Conflict of Interest**
The authors declare no conflict of interest in this work

**Acknowledgement**
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3. The Ministry of Health of Republic Indonesia. Infodatin, Data and information centre of the Ministry of Health of Indonesia, the month of Breast Cancer Care. ISSN. 2016; 2442-7659.


Ethanol Extracts of *Coleus tuberosus* Flesh and Peel as a Potential Source of Natural Antioxidant and Breast Cancer Prevention Agent

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Abstract
This study examines the effects of ethanol extracts of *Coleus tuberosus* flesh and peel on T47D cancer cells. Antioxidant potential was evaluated through cellular antioxidant activity experiment, and anti-proliferation activity was evaluated using MTT assay (3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide). The effects on cell cycle were evaluated with a flow cytometry, while induction of apoptosis was evaluated based on morphological changes by staining with acridine orange and ethidium bromide. The results indicate that the peel extract had higher cellular antioxidant activity than the flesh extract. The IC₅₀ of cellular antioxidant activity of the flesh and peel extracts were 287.13±10.35 µg/ml and 217.86±12.96 µg/ml, respectively. The peel extract also had higher anti-proliferative activity. The IC₅₀ of anti-proliferative activity were 887.05±5.03 µg/ml (flesh extract) and 548.18±4.52 µg/ml (peel extract). The peel and flesh extracts can cause cell cycle arrest in the S phase and G2-M phase. The extracts induce apoptosis within T47D cancer cells, showing an orange color. Therefore, these extracts could be used as potential sources of natural antioxidants and breast cancer prevention agents.

Introduction
Breast cancer is the most common cancer and cause of cancer-related deaths among women, accounting for 1.67 million (25.2%) new cases and 521,907 (14.7%) deaths worldwide¹. Breast cancer is a cancer that develops from the breast tissue. This is an invasive cancer that often appears in women. Some signs of breast cancer are a change of breast shape, dimpled skin, nipple discharge or red scaly patches of skin, and a lump in the breast². Based on data of...
the International Agency for Research on Cancer (IARC) in 2012, the incidence of cancer in women has reached 134 per 100,000 of the population. The highest incidence is breast cancer with 40 cases per 100,000 women followed by cervical cancer reaching 17 cases per 100,000. The estimate of the death toll in Indonesia Globocan for breast cancer is 16.6 deaths per 100,000 population.

Cancer cases are linked to diet and lifestyle. Although surgery and chemotherapy can lower the risks of breast cancer in some women who suffer the disease, the side effects cause them to limit the use. Currently, preventive efforts have become more important. Some natural products have an important role in the discovery and development of drugs for the treatment of various types of deadly diseases including cancer. Several studies have investigated the anti-proliferation effects of some phytochemicals derived from fruits and vegetables. Research examines cancer prevention by utilizing foods that contain bioactive compounds such as antioxidants and antiproliferation, apoptosis effects. More than 1,000 different phytochemicals have been identified as having potential effects against various cancers. These phytochemicals offer considerable advantages because they are safe and may target multiple cell-signaling pathways. Related to the high occurrence of breast cancer in Indonesia, T47D cancer cells are often used as research models to identify the ability of anti-proliferation of bioactive compounds contained in fruits, vegetables, cereals, legume or tubers.

**Coleus tuberosus** is a source of carbohydrate. Some research suggests that it has potential antioxidant and anti-proliferation effects. Bioactive compounds identified in the **Coleus tuberosus** which include phenols, flavonoids, oleanolic acid, ursolic acid, maslinic acid, and phytosterol have antioxidant, and antiproliferation, apoptosis effects. Further research on the antioxidant activity and cancer prevention activity is still needed. This study examines the potential of **Coleus tuberosus** extract as natural antioxidant and breast cancer prevention agents using T47D cancer cells.

**Materials and Methods**

**Chemicals**

This study investigated the effects of ethanol extracts of **Coleus tuberosus** flesh and peel on T47D cancer cell line. MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) (Sigma Aldrich), RPMI (Sigma Aldrich), phosphate buffered saline (PBS) (Gibco), DMSO (Sigma Aldrich), and acridine orange - ethidium bromide (Bio Rad) were used.

**Preparation of Ethanol Extracts of Coleus Tuberosus Flesh and Peel**

The peels of **Coleus tuberosus** were separated with peeler from the flesh with a thickness of 1 mm. The flesh and peel were dried in a cabinet dryer for twenty four hours and then crushed and extracted using maceration with 95% ethanol for 7 days. The extracts were stored in a frozen condition.

**Evaluation of Cellular Antioxidant Activity of Ethanol Extracts of Coleus Tuberosus Flesh and Peel in T47D Cancer Cells**

The cellular antioxidant activity was evaluated by referring to Wolfe and Liu and Liu and Finley. T47D cells grown on a 96-well microplate containing RPMI were supplemented with fetal bovine serum 10% (v/v), penicillin 100 U, and streptomycin 100 mg/ml at 37°C under 5% CO₂. After 24 hours of growth, growth medium was removed, and the wells were washed with sterile PBS. The cells were pretreated with ethanol extracts of **Coleus tuberosus** flesh or peel in concentrations of 100, 200, 400, and 800 µg/ml for 20 mins in triplicate. After 20 mins of pretreatment, growth media containing 25 µM DCFH-DA and PMA (100 ng/ml in DMSO) were added and incubated for 30 minutes. The fluorescence of the cells was measured using a flow cytometer (FACS Calibur BD) at a wavelength of 535 nm. The cellular antioxidant activity was determined by calculating the percentage decrease in the intensity of the reactive oxygen species (ROS) fluorescence:

\[
\text{Decreased ROS of percentage} = \left( \frac{F_{1t2}-F_{1t1}}{F_{0t2}} \right) \times 100\%
\]

where \( F_{0t} \) is the oxidative stress control, \( F_{1t} \) is the cells with bioactive compound treatment, and \( F_{2t} \) is that of the control without oxidative stress.

**Evaluation of Anti-Proliferation of T47D Cancer Cell Lines**

The anti-proliferation effects were evaluated by referring to Hogan et al. Cells were placed on a 96-well plate at 1.5x10⁴ cells/ml with RPMI plus 10% (v/v) fetal bovine serum, penicillin 100 U and
streptomycin (100 mg/ml, 37°C, 5% CO₂). These conditions were kept for a one-hour period. Next, cancer cells were treated for about one hour in the experiment media with ethanol extract of *Coleus tuberosus* peel or flesh extracts at concentrations of 62.5-2,000 µg/ml with the final volume of 100 µl. Cell viability was determined by MTT assay. After one-hour incubation, the media were treated and eliminated at the end of the incubation period and then washed with HBSS. The cells were incubated with 10 µL of MTT reagent solution (5 mg/ml in RPMI) added to each of the wells for 24 hours.

The mixtures were left for one night. The absorbance was recorded at 570 nm with a multipliable plate reader. The absorbance data required for cell viability are expressed as a percentage of the control (the number of living cells in control cells) during the experiment. After each treatment, the MTT assay was expressed as:

\[
\text{Cell viability} = \left( \frac{\text{Absorbance of treatment group}}{\text{Absorbance without treatment}} \right) \times 100\%
\]

**Cell Cycle Arrest**

A total of 10⁶ cell/well were distributed in 6-well plates and incubated at 37°C for cell adaptation. T47D cells incubated with ethanol extract of *Coleus tuberosus* peel or flesh extracts with concentrations of 7.8125, 15.625, 31.25, 62.5 and 125 µg/ml for 24 hours. After 24 hours, cells incubated were taken and washed twice using ice-cold PBS. The cells were fixed and permeabilized with 70% ice-cold ethanol at 4°C for one hour. Then, T47D cells were washed with PBS and resuspended in a solution containing propidium iodide stain (50 µL/ml) and RNase A (250 µg/ml). Cell suspensions were incubated for 30 min at room temperature, followed by fluorescence-activated cell sorting (FACS; cater-plus flow cytometry; Becton Dickinson co., Germany) using 10,000 cells per group. The percentages of cells in the G0-G1, S, and G2/M phases were analyzed using Modfit LT Cell Cycle 3.0 analysis software (Becton Dickinson).

**Induction of Apoptosis**

The morphological changes due to apoptosis induction after treatment with the extracts were evaluated using acridine orange staining and ethidium bromide. T47D cells were cultured on the cover slip on 10⁵ cells/well. The medium was replaced with medium containing ethanol extract samples of *Coleus tuberosus* flesh or peel extracts (62.5 µg/ml). The cells were then incubated for 24 hours at 37°C in 5% CO₂. The medium was removed and solution of acridine orange and ethidium bromide (100 µg/ml of acridine orange (Bio-Rad) in PBS and 100 µg/ml of ethidium bromide (Bio-Rad) in PBS for 5 minutes) was added. The cover slips were mounted on a glass object and then observed under a fluorescence microscope (Zeiss MC 80). The morphology of the cells was inspected under the fluorescence microscope (Zeiss MC 80) and photographed.

**Statistical Analysis**

Each experiment was performed three times and data were expressed as mean ± SD. The testing was performed by two way anova of the antioxidant activity, anti-proliferation effects and cell cycle arrest. If the obtained results are different significantly, then it was followed with Duncan's Multiple Range Test (DMRT).

**Results and Discussion**

**Cellular Antioxidant Activity of Ethanol Extracts of Coleus Tuberosus Flesh and Peel on T47D**

Treatment of T47D cancer cells with ethanol extracts of *Coleus tuberosus* flesh (EECF) or ethanol extract of *Coleus tuberosus* peel (EECP) inhibited PMA-induced ROS generation in dose-dependent manner. The percentage decrease of ROS in cancer cells incubated with peel extract T47D was higher than that of cells incubated with ethanol extract of *Coleus tuberosus* flesh or peel (Fig. 1).

The percentage decreases of ROS in the T47D cancer cells incubated with ethanol extract of *Coleus tuberosus* flesh at concentrations of 100, 200, 400, and 800 µg/ml were 22 ± 7.93, 38.70 ± 5.49, 55.96 ± 2.04, and 62.08 ± 1.54, respectively. The cellular antioxidant activity of peel extract on T47D cells was higher than that of flesh extract, which is shown by the IC₅₀ values. The IC₅₀ of the flesh extract was of 287.13 ± 10.35 µg/ml, whereas that of the peel extract was 217.86 ± 12.96 µg/ml (Table 1).
FIG. 1: The percentage decrease in reactive oxygen species (ROS) with the treatment of ethanol extract of *Coleus tuberosus* flesh and peel on T47D cells induced with phorbol myristate acetate.

Note: Different notations suggest a significant difference (p < 0.05)

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![Graph showing the percentage of viability cells produced by treatments with an ethanol extract of Coleus tuberosus flesh (EECF) and peel (EECP) at different concentrations after a 24-hour incubation. Data are presented as the mean of three independent experiments. Means followed by different letters is significant difference (p < 0.05).](image)

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![Graph showing cell cycle distribution](image.png)

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<tr>
<td>Positive control (apoptotic cells) (B)</td>
<td></td>
</tr>
<tr>
<td>Treatment: 62.5 µg/ml (C)</td>
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</tr>
</tbody>
</table>

Fig. 5: A. Untreated cells, B. treated cells with doxorubicin, C. treatment with concentration of 62.5 µg/ml

**Conclusion**

Ethanol extracts of *Coleus tuberosus* flesh and peel can reduce reactive oxygen species in T47D cells induced with the radical generator PMA. The ability of the peel extract to reduce oxidative stress is better than that of the flesh extract. Incubation of cells with the extracts can reduce proliferation, causing cell cycle arrest in the S and G2-M phases, and induced apoptosis.

**Conflict of Interest**

The authors declare no conflict of interest in this work

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