The Journal of Infection in Developing Countries

← Back to Submissions

16761 / **Ervianti et al.** / Comparison of tea tree oil 5%, tea tree oil 10%, and nystatin inhi Library

Workflow **Publication**

Submission Review Copyediting Production

Submission Files

Search

149558	evy_ervianti, Manuscript TTO VVC JIDC.docx	April 30, 2022	Article Text
149559	evy_ervianti, Figure 1.eps	April 30, 2022	Figure
149560	evy_ervianti, Figure 2.eps	April 30, 2022	Figure
149561	evy_ervianti, Figure 3.eps	April 30, 2022	Figure
149562	evy_ervianti, Figure 4.eps	April 30, 2022	Figure
149563	evy_ervianti, Figure 5.eps	April 30, 2022	Figure
149564	evy_ervianti, Tables.docx	April 30, 2022	Tables
149565	evy_ervianti, Cover Letter.docx	April 30,	Other

2022

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Pre-Review Discussions

Add discussion

Name	From	Last Reply	Replies	Closed
Comments for the Editor	evy_ervianti 2022-04-30 06:30 BST	3mg0036962 2022-05-13 10:12 BST	2	
Revised File	evy_ervianti 2022-11-12 11:24 GMT	-	0	

Dear Editor in-Chief of The Journal of Infection in Developing Countries,

Here we submitted an original research article titled **Comparison of tea tree oil 5%, tea tree oil 10%, and nystatin inhibition zones against vaginal Candida isolates in pregnancy.** This manuscript has not published, in press, or submitted elsewhere in English or any other language, and is not currently being considered for publication elsewhere. This manuscript highlights the inhibitory effects of Tea Tree Oil (TTO) 5% and 10% on Candida species isolated from pregnant patients with vulvovaginal candidiasis, thus might be a potential treatment in the future. All authors have seen and approved the content of the manuscript and have contributed significantly to the work. We do hope that this article could be published in your journal thus this effort would have scientific base as it will be peer-reviewed.

We are looking forward to hearing the good news from you.

Thank you very much.

Kind regards,

On behalf of all Authors,
Evy Ervianti, M.D.
Departement of Dermatology and Venereology
Faculty of Medicine Universitas Airlangga
Surabaya, Indonesia

Comments for the Editor

×Close Panel

Participants Edit

- Evy Ervianty (evy_ervianti)

Messages

Note	From
Dear Editor-in-Chief of The Journal of Infection in Developing Countries, Here we submitted an original research article titled **Comparison of tea tree oil 5%, tea tree oil 10%, and nystatin inhibition zones against vaginal Candida isolates in pregnancy.** This manuscript has not been published, in press, or submitted elsewhere in English or any other language, and is not currently being considered for publication elsewhere. This manuscript highlights the inhibitory effects of Tea Tree Oil (TTO) 5% and 10% on Candida species isolated from pregnant patients with vulvovaginal candidiasis, thus might be a potential treatment in the future. All authors have seen and approved the content of the manuscript and have contributed significantly to the work. We do hope that this article could be published in your journal thus this effort would have a scientific base as it will be peer-reviewed. We are looking forward to hearing the good news from you. Thank you very much. Kind regards, On behalf of all Authors, Evy Ervianti, M.D. Department of Dermatology and Venereology Faculty of Medicine Universitas Airlangga Surabaya, Indonesia	evy_ervianti 2022-04-30 06:30 BST
Dear Evy, M.D. Department of Dermatology and Venereology Faculty of Medicine Universitas Airlangga Surabaya, Indonesia Dear Author the correction of the references necessary before sending the paper for review.	3mg0036962 2022-05-09 20:32 BST

Note From

References Format

JIDC uses the numbered citation method. The references must be listed and numbered consecutively in the order in which they appear in the text followed by those appearing in figures and tables. Citations should be indicated by their unique reference number in square brackets in the text. Where there are multiple citations within a single set of brackets these should be separated by commas with no spaces between the comma and the next number. If there are three or more sequential citations, the numbers should be given as a range. Example: "…….previously described above [1,6-8,26]."

Authors are encouraged to keep the number of references limited to those that are important for the understanding of the manuscript.

JIDC recommends the use of referencing software such as Zotero, which is Free for download at www.zotero.org

If you have any problems using any of this software, please contact the respective company for technical advice.

There are several .csl templates for JIDC style in repositories around the internet, while none of them has been officially made by our developers, and almost all of them are wrong. We encourage authors to download JIDC references style at this link.

Reference format examples:

Published Papers

1. Raghu MB, Deshpande A, Chintu C (1981) Oral rehydration for diarrhoeal diseases in children. Trans R Soc Trop Med Hyg 75: 552-555.

In Press Papers

2. Kharitonov SA, Barnes PJ Clinical aspects of exhaled nitric oxide. Adv Clin Path. In press.

Article within a journal supplement

3. Baquero F, Barrett JF, Courvalin P, Morrissey I, Piddock L, Novick WJ (1999) Epidemiology and mechanisms of resistance among respiratory tract pathogens. Clin Microbiol Infect 4 Suppl 2: 19-26.

Electronic Journal Articles

4. Loker WM (1996) "Campesinos" and the crisis of modernization in Latin America. Jour Pol Ecol 3. Available: http://www.library.arizona.edu/ej/jpe/volume_3/ascii-lokeriso.txt. Accessed 11 August 2006.

Books

Whole Book

5. Lucas AO and Gilles HM (2003) Short textbook of public health medicine for the tropics, 4th edition. London: Arnold Press 389 p.

Note From

Book Chapters

6. Fernández E and Torres AC (2006) Gender differentials in health. In Jamison DT, Bremen JG, Measham AR, Alleyne G, Cleason M, Evans DB, Jha P, Mills A, Musgrove P, editors. Disease Control Priorities in Developing Countries. New York: Oxford University Press. 195-210.

Accession Numbers

We encourage authors to deposit relevant datasets, images, nucleotide and protein sequences and microarray data in public resources. The relevant accession numbers and where appropriate the version numbers of such deposited material should be mentioned. Suggested databases include, but are not limited to

-Microarray data: ArrayExpress ; Gene Expression Omnibus [GEO]
-Nucleotide sequences: DNA Data Bank of Japan [DDBJ] ; European Molecular Biology Laboratory (EMBL/EBI) -Nucleotide Sequence Database, or GenBank (National Center for Biotechnology Information).
-Protein sequences: UniProtKB/Swiss-Prot; Protein Data Bank
-Computional modeling: BioModels Database
-Plasmids: Addgene, or PlasmID,Database of Interacting Proteins
-Chemical structures and assays: PubChem Substance; PubChem BioAssay
"-Multilocus sequence typing data for bacteria: www.mlst.net

Manuscript General Requirements

Abbreviations

Abbreviations must be defined when they are first used in the text.

Nomenclature & Taxonomy

JIDC recommends the use of correct and established nomenclature wherever possible:

- SI units should be used throughout
- Genus and Species names should be italicized (*e.g.*, *Plasmodium falciparum*). Where the genus appears in the title it should be written out in full. In the main text, the genus should be written out in full at first mention and thereafter abbreviated *e.g.* (*P. falciparum*). Authors must ensure that there is no confusion with other genera mentioned in the text. The spelling and taxonomy of names of microorganisms should follow internationally accepted nomenclature.
- Genes, mutations, genotypes, and alleles should be italicized. Authors should consult appropriate genetic nomenclature databases (*e.g.*, HUGO) for human genes for the recommended names. Proteins are not normally italicized.
- The Recommended International Non-Proprietary Name (rINN) of drugs should be provided. Commercial names of other products should only be used where there is no other suitable term for the product. In such cases, the name, city and country of the manufacturer should be provided in parenthesis at the first mention of the product.

Attach the file to this correct conversation within 5 days.

Note
Best regards

Matthew Donadu, PhD , JE
reminder comunication

From

3mg0036962
2022-05-13
10:12 BST

Dear Evy, M.D.

Department of Dermatology and Venereology

Faculty of Medicine Universitas Airlangga

Surabaya, Indonesia

Dear Author

the correction of the references necessary before sending the paper for review.

References Format
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Note From

Article within a journal supplement

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Electronic Journal Articles

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Accession Numbers

We encourage authors to deposit relevant datasets, images, nucleotide and protein sequences and microarray data in public resources. The relevant accession numbers and where appropriate the version numbers of such deposited material should be mentioned. Suggested databases include, but are not limited to

-Microarray data: ArrayExpress ; Gene Expression Omnibus [GEO]
-Nucleotide sequences: DNA Data Bank of Japan [DDBJ] ; European Molecular Biology Laboratory (EMBL/EBI) -Nucleotide Sequence Database, or GenBank (National Center for Biotechnology Information).
-Protein sequences: UniProtKB/Swiss-Prot; Protein Data Bank
-Computional modeling: BioModels Database
-Plasmids: Addgene, or PlasmID,Database of Interacting Proteins
-Chemical structures and assays: PubChem Substance; PubChem BioAssay
"-Multilocus sequence typing data for bacteria: www.mlst.net

Manuscript General Requirements

Abbreviations

Abbreviations must be defined when they are first used in the text.

Nomenclature & Taxonomy

JIDC recommends the use of correct and established nomenclature wherever possible:

- SI units should be used throughout
- Genus and Species names should be italicized (*e.g.*, *Plasmodium falciparum*). Where the genus appears in the title it should be written out in full. In the main text, the genus should be written out in full at first mention and thereafter abbreviated *e.g.* (*P.*

Note From

falciparum). Authors must ensure that there is no confusion with other genera mentioned in the text. The spelling and taxonomy of names of microorganisms should follow internationally accepted nomenclature.

- Genes, mutations, genotypes, and alleles should be italicized. Authors should consult appropriate genetic nomenclature databases (*e.g.*, HUGO) for human genes for the recommended names. Proteins are not normally italicized.
- The Recommended International Non-Proprietary Name (rINN) of drugs should be provided. Commercial names of other products should only be used where there is no other suitable term for the product. In such cases, the name, city and country of the manufacturer should be provided in parenthesis at the first mention of the product.

Attach the file to this correct conversation within 1 day.

Best regards

Matthew Donadu, PhD , JE

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Revised File

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Participants Edit

- Marina Pekmezovic (pandamaki)
- Matthew G. Donadu, PhD (3mg0036962)
- Evy Ervianty (evy_ervianti)

Messages

Note	From
Dear Editor of JIDC. We apologize for our delay in responding your email. Here we attach our revised article as suggested by the editor. Hopefully our article can proceed to the next stage (peer reviewer) Regards, Evy Evianty, MD evy_ervianti, NASKAH FULL (1).doc	evy_ervianti 2022-11-12 11:24 GMT

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The Journal of Infection in Developing Countries

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16761 / **Ervianti et al.** / Comparison of tea tree oil 5%, tea tree oil 10%, and nystatin inhi Library

Workflow | Publication

Submission | Review | Copyediting | Production

Round 1 | Round 2

Round 1 Status
Submission declined.

Review Discussions

Add discussion

Name	From	Last Reply	Replies	Closed
Revised File	evy_ervianti 2022-12-14 13:56 GMT	evy_ervianti 2022-12-31 07:00 GMT	4	

Revised File

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Participants Edit

- Marina Pekmezovic (pandamaki)
- Matthew G. Donadu, PhD (3mg0036962)
- Evy Ervianty (evy_ervianti)

Messages

Note
Manuscript ID: #16761

From
evy_ervianti
2022-12-14
13:56 GMT

Dear Editor,

Thank you for giving us the opportunity to revised our manuscript entitled "**Comparison of tea tree oil 5%, tea tree oil 10%, and nystatin inhibition zones against vaginal Candida isolates in pregnancy"** based on the suggestion from reviewer. We appreciate the dedicated time and effort from you and reviewers. We already revised based on the reviewer's comment. Those change are highlighted in this manuscript. Please the attached file. Thank you.

Regards,

Author

evy_ervianti, REVISI 1.doc evy_ervianti, REVISI TABEL.docx

Dear Author,

please upload the revised files in the Journal system.

pandamaki
2022-12-19
14:29 GMT

Best,

Marina
Settings

Note	From
Dear Editor, The revised file already attached in previous discussion in journal system. Should it be attached again? Best regard, Author	evy_ervianti 2022-12-28 01:54 GMT
Please do not attach the files in the discussion, upload it via system (same as for submitting the manuscript). Additionally, please include "Response to reviewers" file. Thank you!	pandamaki 2022-12-29 11:06 GMT
Settings Dear Editor, We already attached our revised file in Revisions Sub-Menu. Should it be uploaded as a new submissions again? Kindly waiting for your assistance. Thank you Regards, Editor	evy_ervianti 2022-12-31 07:00 GMT

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16761 / **Ervianti et al.** / Comparison of tea tree oil 5%, tea tree oil 10%, and nystatin inhi **Library**

Workflow **Publication**

Submission Review **Copyediting** **Production**

Round 1 Round 2

Round 2 Status
Submission accepted.

Notifications

[JIDC] Editor Decision 2022-05-17 21:11 BST

The Journal of Infection in Developing Countries

← Back to Submissions

[JIDC] Editor Decision 2023-04-01 07:54 BST

Reviewer's Attachments

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Revisions

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▸	165082	evy_ervianti,+REVISI+1.doc	December 31, 2022	Article Text

▶	165083 evy_ervianti,+REVISI+TABEL.docx	December 31, 2022	Tables	
▶	165084 Response to reviewer.docx	December 31, 2022	Other	

Review Discussions

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Name	From	Last Reply	Replies	Closed
▶ Revised File	evy_ervianti 2022-12-14 13:56 GMT	evy_ervianti 2022-12-31 07:00 GMT	4	☐

Notifications
×undefined
[JIDC] Editor Decision
2022-05-17 21:11 BST

Dears

Evy Ervianty, Damayanti, Indah Purnamasari, Linda Astari, Budi Prasetyo, Pepy D. Endraswari, Budi Utomo, Endang Wahyu Fitriani, Diah Mira Indramaya, M. Yulianto Listiawan, Cita Rosita Sigit Prakoeswa:

An initial review of your recent submission to the Journal of Infection in Developing Countries, has made it clear it can't be accepted, due to missing basic technical requirements.

You are kindly requested to carefully check the following document: https://jidc.org/index.php/journal/about/submissions use the MS Word manuscript template provided, and review the following aspect(s) accordingly:

You are welcomed to resubmit your work, once it is compliant to the guidelines and instructions provided, starting a new submission.

--

I inform you that the wait for publication is very long, if the paper passes the review process, it would be at least 8 months.

If you prefer, you can submit your paper to
the https://www.hindawi.com/journals/cjidmm/ or www.jmidonline.org

Please do not hesitate to contact me for any additional information.

Best Regards,

==========

* a properly formatted cover letter was not submitted.

* the submitted cover letter should declare that this manuscript is original, is not published, in press, or submitted elsewhere in English or any other language and is not currently being considered for publication elsewhere.

* the submitted cover letter should declare that all authors have seen and approved the content of the manuscript and have contributed significantly to the work.

* a standard 12 points Serif font should be consistently used for the manuscript text.

* your submission's Abstract is 000 words long, exceeding the maximum allowed length of 250.

* a properly formatted Title Page for your submission, including the title of the manuscript as well as the full names and institutional affiliations for all authors, abstract, keywords, and a Running Title, is missing. It should be placed at the beginning of your manuscript file.

* the Title Page is not properly formatted. It should include the title of the manuscript as well as the full names and institutional affiliations for all authors (organised in two separated lists connected by superscript numbers), abstract, keywords, and a Running Title. It should be placed at the beginning of your manuscript file.

* your submission's title is 000 characters long (including spaces), exceeding the maximum allowed length of 125.

* according to the category you've chosen for your submission, the abstract should be divided into the following sections: introduction, methodology, results, conclusions.

* your submission's abstract is xxx words long. Exceeding the maximum allowed length of 250 words.

* your submission's Running Title is 000 characters long (including spaces), exceeding the maximum allowed length of 50.

* a running title of no more than 50 characters (including spaces) was not provided.

* all submitted items should be properly Left-To-Right formatted.

* your article is 0000 words long excluding references and title page, which is too short to be submitted as an Original Article (2100 words long). It should have been submitted as a Brief Original Article.

* when used in the context of a word processing text, the term "double spaced" refers to the spacing between lines, not words. Please make sure your manuscript text is correctly formatted without unnecessary spaces between words, and uses a standard 12 point serif font.

* Microsoft Word proprietary footnotes format is not accepted. References and bibliography should be provided as inline text.

* References within the text and in the references section are not properly formatted. Examples are provided in the Author Guidelines (https://www.jidc.org/index.php/journal/about/submissions), in the aforementioned manuscript template, and all the previously published articles. To easily gather and properly fit references into your manuscript, you may want to use Zotero, which is free software available at www.zotero.org.

* Please note that the “et al.” formula is not allowed in the references section according to the Journal’s style for bibliography. Please use all authors’ names.

*References are only accepted in English. If the text is not available in English, the following example of citation is provided in the guidelines:

2. Abdon NP, Pinto AYN, Silva RSU, Souza JM (2001) Assessment of the response to reduced treatment schemes for vivax malaria. Rev Soc Bras Med Trop 33: 343-348. [Article in Portuguese]. The title must be translated in English. If the text is available in English, please provide the citation for the English version.

*In the references section Journal names must be used in their official abbreviation version. You can look for abbreviations at the link: http://www.journalabbr.com or in the journals' websites.

*References numbers should be placed at the end of the sentence before the dot.

* manuscripts including tables or images are not accepted. All tables should be submitted in a single supplementary file, MS Word .doc or .docx file format, with one table per page, i.e. ONE file with ALL tables in it. this may seem insignificant to you, but not all the JIDC reviewers have broadband internet access, and having to download many files can be really time and resources consuming.

* all submitted tables must be proper ones, i.e. use of formatting symbols like ENTER, TAB or SPACE to separate lines or columns within cells is not acceptable. Make sure you use the TABLE tool of your text editor, and properly place data into cells, rows and columns, keeping formatting to the lowest possible.

* the table you submitted is not properly formatted, i.e. rows and columns are inverted, using a text direction hack. This makes the whole table unsuitable for printing purposes. It should be redone by placing all items in the correct rows/columns order.

* in your manuscript text and tables, you have used several times the symbols "±", "≤" and "≥", but instead of properly placing them, you have typed the simple "+" "<" and ">" sign, using the underline text format to draw a line under it or placed a symbol followed by the "=".

This is perfect for the human eye, since the brain can interpret the symbol correctly but, once in a scientific document, the two symbols have completely different meaning.

* images included in MS word documents are not accepted. Images must be uploaded as separated supplementary files, tiff, pdf, or eps format, at a resolution suitable for print, and they must adhere to PubMed Central specifications.

* the images you submitted are of very poor quality and at an insufficient pixel resolution.

Please check the PMC image table at:
http://www.ncbi.nlm.nih.gov/pmc/pub/filespec-images/#fig-format
for reference, and provide new original image files according to the correct graphic type.

=> IMPORTANT: please avoid trying to "adjust", "improve", "convert" or "fix" the images you already uploaded. There is no workaround, since by now the quality has been compromised by the compression applied: new images have to be produced from scratch.

You may want to check the following guide from PLoS on how to properly export and save your image files:
http://journals.plos.org/plosntds/s/figures

See if your software of choice is listed, and follow the instructions. If it's not there, consider that almost all latest versions of analysis and drawing programs have more than an option to save high resolution formats, while it could be tricky sometimes. Refer to your software manual for details.

To properly format your graph, please follow the procedure shown in the following video:
http://vimeo.com/35497771
if you are in trouble with the format conversions, just *send the powerpoint .pptx file*

I see you have used the correct procedure to create the graphs, but it's the Word format which does not allow to print high resolution images. Please Use PowerPoint instead (same procedure) to generate graphs, and upload the PowerPoint .pptx file.

When dealing with maps, the best starting point is a blank vector file, as the ones you can access from this Wikipedia Page:

http://commons.wikimedia.org/wiki/Category:SVG_maps_by_country

(remember to properly cite the source)

*figures' legends should not be part of the figures themselves but they should be placed at the end of the article text.

*composed figures must be submitted in a single file including all figure's parts and compliant with PubMed requirements in all its parts.

* We strongly recommend authors whose native language is not English to have their manuscript checked by a language editing service, or by an English native speaker colleague prior to submission. In the submitted manuscript there are typos and not fully correct sentences, for this reason we cannot accept it.

*We suggest revising the manuscript's style and to use impersonal style. Please read this guide about academic writing: http://library.bcu.ac.uk/learner/writingguides/1.20.htm

* authors' names and ordering in the submission metadata section do not match the information provided in the manuscript title page. Please acknowledge

* In step 3 of the submission process, you are required to provide complete, ordered, consistent and correctly formatted metadata information for all authors participating in the research. Please fill in all first and last names Capitalizing First Letters and avoiding abbreviations, correctly and extensively indicate the respective affiliations and Country names.

==>> IMPORTANT: PubMed records are compiled from the metadata page, so it should be in your best interest to make sure this information is the most accurate and clear as possible. Exact First and Last Names spelling, complete (not abbreviated) institution names and authors' email contacts and order are under your responsibility.

Since providing correct spelling for your name is your responsibility, make sure you double-check every change it may happen on the manuscript is also reported in the Metadata section throughout the submission review and editing process, should it happen you make changes in the abstract text or add/remove authors.

*please fill in the attached metadata form, it will be my care to edit the metadata.

*the abbreviations list is not compliant with the Journal's style. Please indicate the abbreviations' meaning at the first time that they are mentioned in the article text.

*the manuscript is not properly formatted. Please avoid including: header, footer, footnotes, page numbers, paragraph numbers, lines, colours, and bold where not requested by the Journal's style. Also avoid to include outlines and abbreviation list.

* Figure legends must be placed at the end of the article text and not be part of the figures themselves.

*Figures' legends or tables' captions should be placed at the end of the article text and not in the middle of it.

*Figure legends for composed figures must be organized as a single figure legend divided into the different parts. I.e.: figure X: ..... (a)..... (b).....

We have reached a decision regarding your submission to The Journal of Infection in Developing Countries, "Comparison of tea tree oil 5%, tea tree oil 10%, and nystatin inhibition zones against vaginal Candida isolates in pregnancy".

Our decision is to: Decline Submission

Matthew G. Donadu, PhD
University of Sassari, Department of Chemistry and Pharmacy
mdonadu@jidc.org

Journal Editor

--

The Journal of Infection in Developing Countries

Notifications
×undefined
[JIDC] Editor Decision
2022-12-09 05:26 GMT

Evy Ervianty, Damayanti, Indah Purnamasari, Linda Astari, Budi Prasetyo, Pepy D. Endraswari, Budi Utomo, Endang Wahyu Fitriani, Diah Mira Indramaya, M. Yulianto Listiawan, Cita Rosita Sigit Prakoeswa:

We have reached a decision regarding your submission to The Journal of Infection in Developing Countries, "Comparison of tea tree oil 5%, tea tree oil 10%, and nystatin inhibition zones against vaginal Candida isolates in pregnancy".

Our decision is: Major Revision.

The reviewers suggested major revisions to your manuscript. Therefore, I invite you to respond to the reviewers' comments and revise your manuscript.

Revise your manuscript using a word processing program and save it on your computer. Please also highlight the changes to your manuscript within the document by using the track changes mode in MS Word or by using bold or coloured text.

Once the revised manuscript is prepared, you can upload it and submit it through your Author Center.

When submitting your revised manuscript, you are requested to highlight the changes in the manuscript. Moreover, you are definitely requested to prepare a cover letter stating in details which changes have been made corresponding to the individual suggestions by the various peer reviewers. In case you might come to the conclusion that a particular suggestion should not be followed in full or to some extent please do provide an explanation for your decision in the cover letter.

The revised manuscript will be sent out for further peer review so we cannot guarantee acceptance at this stage.

Once again, thank you for submitting your manuscript to The Journal of Infection in Developing Countries and I look forward to receiving your revision.

Dr. Marina Pekmezovic
marinapekmezovic@gmail.com

Reviewer 1

The manuscript **Comparison of *tea tree oil* 5%, *tea tree oil* 10%, and nystatin inhibition zones against vaginal *Candida* isolates in pregnancy** is well conceptualized with well-defined research objectives. Methods are detailed and all figures and tables in the paper properly represent the results. Accept after minor revision: add nystatin concetration in Methods; *in vitro* write italic, and check check for spelling mistakes there are many.

Recommendation: Accept Submission

Reviewer 2:

GENERAL

Suggested to cross-check all values quoted in article against those quoted in Figures

INTRODUCTION

1. Spelling error : Last Paragraph
    1. Acitivity

METHODOLOGY

1. Microbiological assay
    1. Specify the media used for antifungal susceptibility testing in this study

RESULT

1. Table 2 : Nystatin inhibition zone
    1. Recheck the interpretation for resistant (whether no zone of inhibition or < 10mm)
2. Second paragraph :

Your sentence: Seven (38.8%) of the isolates were *C. albicans* while eleven (11) isolates were non-*albicans* (61.6%) species, consisting of *C. glabrata* (54.4%), *C. dubliniensis* (36.3%) and *C. parapsilosis* (18.1%) as shown in **figure 2** and **figure 3**.

Clarification:

(11) isolates were non-*albicans* **(61.6%)**

>> This percentage do not tally with the figure where non-albicans quoted at **61.11%**

For non-albicans isolate (***glabrata*** **(54.4%),** *C. dubliniensis* (36.3%) and *C. parapsilosis* (18.1%)) total up to 108.8%.

>> Candida glabrata quoted at **45.45% in** Figure 2 which do not tally with text

DISCUSSION

1. First paragraph
    1. Your sentence : . They also have a possible low vaginal defense mechanism against *Candida* species and have increased levels of estrogen and corticoids and, therefore, more **resistan**t to *Candida* species infections

Correction: high level of estrogen and corticoid make them more **SUSCEPTIBLE** to Candida infection

Recommendation: Revisions Required

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The Journal of Infection in Developing Countries

Notifications
×undefined
[JIDC] Editor Decision
2023-01-16 09:13 GMT

Evy Ervianty, Damayanti, Indah Purnamasari, Linda Astari, Budi Prasetyo, Pepy D. Endraswari, Budi Utomo, Endang Wahyu Fitriani, Diah Mira Indramaya, M. Yulianto Listiawan, Cita Rosita Sigit Prakoeswa:

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Comparison of *tea tree oil* 5%, *tea tree oil* 10%, and nystatin inhibition zones against vaginal *Candida* isolates in pregnancy

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Abstract
Introductions: Vulvovaginal candidiasis (VVC) in pregnancy frequently develops into recurrent infections. Clinical study suggests that conventional topical treatments for VVC is not always enough to eradicate *Candida* spp. from the vaginal microenvironment. This study aimed to evaluate the antifungal activity of *tea tree oil* (TTO) 5% and TTO 10% against *Candida* species causing VVC in pregnancy.
Methodology: *In vitro* experimental study was conducted in the Mycology Laboratory at Dermatovenereology Outpatient Clinic Dr. Soetomo General Hospital Surabaya. Eighteen isolates of *Candida* species were isolated from the vaginal thrush of 15 pregnant women diagnosed with VVC from March to May 2021. Antifungal susceptibility of TTO 5%, and TTO 10% was evaluated by the disc diffusion method, with the inhibitory zone diameter as the main outcome.
Results: The mean inhibitory zone diameter of TTO 5%, TTO 10%, and nystatin against all *Candida* spp. was 7.26 mm, 8.64 mm, and 25.57 mm, respectively ($p<0.001$). The mean inhibitory zone diameter of TTO 5%, TTO 10%, and nystatin tend to be larger in *C. albicans* compared to the non-*albicans*, but the difference is not significant. Nystatin displayed the largest mean inhibitory zone diameters compared to TTO 5% and TTO 10% ($p<0.001$) in all *Candida* species. Increased concentration from TTO 5% to TTO 10% resulted in a slight increment in the mean inhibitory zone diameters in all-*Candida* species ($p=0.001$).
Conclusions: *Tea Tree Oil* displayed antifungal activity against *Candida* species causing VVC in pregnancy. Further studies are required to investigate optimal TTO concentrations as a VVC treatment in pregnancy.
Running title: *Tea tree oil* antifungal activity against *Candida*.
Keywords: vulvovaginal candidiasis, pregnancy, *tea tree oil*, nystatin, inhibition zone

INTRODUCTION:

One of a common gynecological problem is vulvovaginal candidiasis (VVC), and in pregnant women, the incidence of VVC is almost doubled [1]. Candidiasis in pregnancy often results in a less effective response to treatment [2]. The incidence of VVC in Dr. Soetomo General Academic Teaching Hospital was still relatively high, with 16 (53%) of the 30 smear samples collected from the posterior vaginal fornix of pregnant women tested positive for *Candida* species [3]. Complications of VVC in pregnancy have been linked to an increased risk of prematurity and low birth weight and significant discomfort to the mother, including redness, increased discharge, burning sensation in the vulva area, and itching [1], [4].

According to epidemiological and clinical statistics, topical therapy does not effectively remove *Candida* infection in the vaginal environment, and about 13% of patients develop recurrent VVC that develop into chronic infections [5]. As many as 5 of 16 women will relapse. Due to the uncomfortable nature, most women employ natural products or drugs that are not licensed for treatment or relapse prevention [6], [7].

Essential oils from some plants, such as *Melaleuca alternifolia*, have been studied for their *in vitro* microbicidal actions and their ability to decontaminate the vaginal canal and prevent fungal colonization by combining fungicidal action [6]. *Tea tree oil* significantly inhibits de novo biofilm formation. In many countries, TTO-containing medications are used to treat vaginal infections and are specially manufactured to be administered intravaginally [8], [9]. However, there were no studies on the effectiveness of TTO in treating its VVC during pregnancy in Indonesia. Therefore, it is crucial to develop new antifungal agents that broaden its spectrum on against *Candida* spp. and become an alternative combination option between conventional and standard antifungal agents in the treatment of VVC in pregnancy. This study aims to asses the antifungal activity of TTO 5%, TTO 10% compared with nystatin against *Candida* spp. causing VVC in pregnancy.

MATERIAL AND METHODS:

Subjects

This study were carried out *in vitro*. Study design have been approved by Ethics Committee of Dr. Soetomo General Academic Teaching Hospital Surabaya. The study protocol was explained to subjects wishing to participate in this study by providing study clarification. Patients gave their consent prior to the study. Samples were collected during 3 months period between March 2021 to May 2021 using total sampling method, and 15 pregnant patients attending Obstetrics and Gynecology outpatient clinic were diagnosed with VVC. Inclusion criteria of subjects were patients diagnosed with VVC having at least one of the VVC symptoms (vaginal discharge like cheese or cracked milk, vaginal itching, erythema, dyspareunia) and fungal positivity (*pseudohyphae* and/or *blastospores*) by Gram staining smears. VVC pregnant patients treated with antifungal therapy, intravaginally or systemically, within the last 2 weeks and with no colony growth on *CHROMagar* media were excluded from the study.

Clinical isolate

Two swabs were taken simultaneously from each patient and immediately sent to Mycology Laboratory at Clinical Microbiology Laboratory of Dr. Soetomo General Hospital Surabaya. The 1st swab was used to make thin smears on microscopic slides for Gram staining and the second swab was cultured on *CHROMagar* media. The cultures were cultivated for 36-48 hours before species identification. In this study, 18 isolates of *C. albicans* species and non-*albicans* were found. The susceptibility of antifungal was assesed by the disc diffusion method on *Mueller Hinton* agar with 2% glucose and methylene blue. *Candida* spp. isolates were plated on agar and paper discs containing TTO 5%, TTO 10%, and nystatin were placed on the media. The cultures were incubated as long as 24-48 hours, and the inhibition zone was measured with a Vernier calipers. The diameter of zones of inhibition between TTO 5%, TTO 10%, and nystatin were then compared.

RESULTS:

A total of fifteen (15) subjects were assigned for isolation and identification of *Candida* species and antifungal susceptibility test. Subjects' characteristics are presented in **table 1**. The majority of subjects were in the 3rd trimester of pregnancy (11; 73.3%), followed by the 2nd trimester of pregnancy (4; 26.7%). No subject was in the 1st trimester of pregnancy as shown in **figure 1**.

Table 1.

Figure 1.

Among the 18 vaginal isolates collected from pregnant women with vulvovaginal candidiasis (VVC), four *Candida* species were isolated and identified: *Candida albicans, Candida glabrata, Candida dubliniensis,*and *Candida parapsilosis.* Seven (38.8%) of the isolates were *C. albicans* while eleven (11) isolates were non-*albicans* (61.1%) species, consisting of *C. glabrata* (45.4%), *C. dubliniensis* (36.3%) and *C. parapsilosis* (18.1%) as shown in **figure 2** and **figure 3**. More than one species of *Candida* was found in 3 subjects.

Figure 2.

Figure 3.

All *Candida* species showed growth inhibition in TTO 5%, TTO 10%, and nystatin. The mean inhibitory zone diameter of TTO 5% in all isolates was 7.26 mm, while *C. albicans* and non-*albicans* had a 7.77 mm and 6.85 mm mean inhibitory zone diameters, respectively. The mean inhibitory zone diameter of TTO 10% in all isolates was 8.64 mm, while *C. albicans* and non-*albicans* had a 9.01 mm and 8.36 mm mean inhibitory zone diameters, respectively. Compared to TTO 5%, TTO 10% displayed slightly larger mean inhibitory zone diameters in all-*Candida* species but was statistically significant (p=0.001). However, we could not determine TTO sensitivity because there were no Clinical and Laboratory Standards Institutes (CLSI) criteria for TTO.

The mean inhibitory zone diameter of nystatin in all isolates was 25.57 mm, while the mean inhibitory zone diameters in *C. albicans* and non-*albicans* was 25.94 mm and 25.29 mm, respectively. Sensitivity criteria according to CLSI for nystatin was used to determine *Candida*

sensitivity **(Table 2)**. According to the criteria, all isolates were sensitive to nystatin, and no strains were resistant to it.

Table 2.

In all *Candida* species, the mean diameter of nystatin inhibition zone was larger than TTO 5% and TTO 10%, which was 25.57 mm compared to 7.26 mm and 8.64 mm, respectively **(Figure 4)**. Kruskal-Wallis tests revealed a significant difference between the diameters of the inhibitory zone between TTO 5%, TTO 10%, and nystatin ($p<0.001$), whereby nystatin was superior to both TTO 5% and TTO 10% in both *C. albicans* and non-*albicans.* In this study, we found that the mean inhibitory zone diameters of TTO 5%, TTO 10%, and nystatin was slightly larger in *C. albicans* compared to *C.* non-*albicans*, but the difference was not statistically significant **(Figure 5).**

Figure 4.

Figure 5.

DISCUSSION:

According to previous reports, one-quarter of all women are exposed to vaginal candidiasis during their lives [11]. This study revealed a total of 15 pregnant women with vulvovaginal candidiasis (VVC) during three months period from March 2021 to May 2021 in Dr. Soetomo General Hospital. The prevalence of VVC in this study was at a higher frequency within the age range 26-35 years (80%) because women in this age range are younger and sexually active. Moreover, some women in this age range are becoming more desirous of having children. They also have a possible low vaginal defense mechanism against *Candida* species and have increased levels of estrogen and corticoids and, therefore, more susceptible to *Candida* species infections [11]. This study found that the women in the 3rd trimester had the highest prevalence rate of VVC. In 3rd trimester pregnant women, symptomatic recurrences are more likely, and therapeutic response is diminished [12]. The immune system of pregnant women in the 3rd trimester of pregnancy is weakened compared to in the 2nd and 1st trimesters, increasing the chance of *Candida* species becoming pathogenic. Vaginal colonization and symptomatic vaginitis are more common during pregnancy. These factors contributed to the highest prevalence of VVC in the 3rd trimester of pregnancy.

Candida albicans is the most common cause of fungal infections of the reproductive tract in women of childbearing age [13]. However, there have been reports in recent years of *Candida* non-*albicans* species being identified from VVC patients, especially *Candida glabrata*, which increasingly being identified as the infection's source [14,15]. Our findings showed that the most common *Candida* species isolated from vaginal discharge was non-*albicans* there are *C. glabrata* (54.4%), followed by *C. dubliniensis* (36.3%), and *C. parapsilosis* (18.1%). Similar study reported by Nelson et al. that *C. glabrata* was the most common cause of VVC in pregnant women [16]. The shifting towards *C.* non-*albicans* as the cause of VVC is concerning for pregnant women, as it has the potential to make VVC chronic, recurrent, and more resistant to antifungal drugs than *C. albicans.* The high resistance levels of *C.* non-*albicans* species to routinely used medications, together with an increase in their identification in women with VVC,

emphasizes the need to identify *Candida* species in vaginal samples to give clinicians information about the best treatment for their patients.

Tea tree oil (TTO) at a concentration of up to 20% was considered safe and without major adverse effects [17]. This study used TTO with 5% and 10% concentrations. This concentration was chosen to prepare the medication for future human use by using the minimal concentration that was effective with low toxicity risk. TTO 5% and TTO 10% showed antifungal activity against *Candida* species in this study. This effect was proved by the TTO inhibition produced in the media with a diameter of 7.26 mm and 8.64 mm in all *Candida* species. According to Hammer et al., TTO and their components improved membrane cell permeability, and this could inhibit the growth of *Candida* [18]. *Tea tree oil* could also produce membrane lipid bilayers that change the component keeping the membrane intact. These changes lead to inhibition of the growth of *Candida* [19]. The mean diameter of TTO inhibition zone of *C.* non-*albicans* were slightly lower than *albicans* species meaning that the antifungal susceptibility of *C. albicans* was better than those in *C.* non-*albicans.*

Non-*albicans* candidiasis is more likely among those with diabetes mellitus, elderly, prior antifungal drugs, or a low socioeconomic status. The pathogenic mechanisms of *C.* non-*albicans* are less well understood than those of *C. albicans*, where more extensive research has been carried out [20]. Intrinsic resistance or low dose susceptibility to azole antifungals, the first-line treatment, is a prominent feature of non-*albicans* species, which leads to treatment failure [21]. Identification and antifungal susceptibility testing are required for optimal treatment in pregnant women of these infections, especially in settings where diagnosis is based on clinical presentation or limited laboratory testing.

The inhibitory zone of nystatin was significantly higher than TTO in diameter. This could be due to different drugs mechanisms. Nystatin was discovered to bind ergosterol, which is the primary component of the fungal cell membrane. It created a pore-like structure, which allowed plasma to flow out, resulting in fungal cell death [22]. This was different from the TTO mechanism, which increased the permeability of the fungal cell membrane but did not form a pore-like structure like it happened in nystatin. *Tea tree oil* was fungistatic, but only at higher concentrations did it become fungicidal [23]. *Tea tree oil* has also been shown to inhibit biofilm formation. The ability of *C. albicans* to adhere and form a biofilm is very important in the incidence of VVC. The antifungal activity of TTO and *terpinen-4-ol* was able to control the proliferation of biofilms *in vitro* [24,25]. On the other hand, nystatin lacks this mechanism. Therefore, TTO can be considered as adjuvant therapy for VVC in pregnancy.

In both *C. albicans* and *C.* non-*albicans*, there was a statistically significant difference in the inhibitory zone diameters of TTO 5% and TTO 10% compared to nystatin ($p<0.001$). The inhibitory zone diameters of *C. albicans* were slightly larger than *C.* non-*albicans*, but were not statistically different. Based on this result, further study of TTO antifungal activity was needed to establish the optimal concentration of antifungal treatment of VVC in pregnancy. *Tea tree oil* must be used in higher concentrations to prevent *C. albicans* from growing [26]. This could be due to the ability of *C. albicans* to form a germination tube and biofilm, which would protect the fungi from TTO-induced environmental changes [27]. *Tea tree oil* demonstrated fungistatic effects, but only at higher concentrations did it have a fungicidal effect (26). In our study, we found significant difference in the inhibition zone diameter between TTO 5% and TTO 10% for each species of *C. albicans* and *C.* non-*albicans*, but the increment in antifungal activities with increasing TTO concentrations were minimum. Given the risks of administering TTO, especially during pregnancy, a concentration of TTO 5% may be sufficient to offer a therapeutic effect on *Candida* that causes VVC.

According to this study, our opinion is that combining TTO with standard medications such as nystatin might be helpful to treat chronic VVC or VVC in pregnancy. Further studies are required to determine the antifungal activity of natural medicinal components and to uncover synergistic interactions with routinely used antifungal drugs.

ACKNOWLEDGEMENT:

The authors would like to thank the Faculty of Medicine, Universitas Airlangga for research funding under the scheme of Penelitian Unggulan Fakultas with the reference number 212/UN3/2021.

CONFLICT OF INTEREST:

All authors have no conflict of interest.

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Table 1. Demographic data of subjects

No	Basic Data			
	Category	**Group**	**N**	**%**
1	Age	Late teens (18 – 25 year)	2	13.3
		Early adult (26 – 35 year)	12	80
		Late adult (46 – 55 year)	1	6.7
		Early senior adult (46 – 55 year)	0	0
		Late senior adult (56 – 65 year)	0	0
2	Education	Elementary school	0	0
		Junior high school	3	20
		High school	5	33.3
		Diploma/bachelor	7	46.7
3	Occupation	Housewives	10	66.7
		Private sector employee	2	13.3
		Government employee	1	6.7
		Nurse	2	13.3

Table 2. Nystatin inhibition zone for *Candida* species (10).

Drug	Concentration	Inhibition Zone Diameter (mm)		
		Sensitive	Intermediate	Resistant
Nystatin	100 U/disc	≥ 15	10-14	≤ 10

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Original Article

Comparison of tea tree oil 5%, tea tree oil 10%, and nystatin inhibition zones against vaginal *Candida* isolates in pregnancy

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Abstract

Introduction: Vulvovaginal candidiasis (VVC) in pregnancy frequently develops into recurrent infections. Clinical study suggests that conventional topical treatments for VVC are not always enough to eradicate *Candida* spp. from the vaginal microenvironment. This study aimed to evaluate the antifungal activity of *tea tree oil* (TTO) 5% and TTO 10% against *Candida* species causing VVC in pregnancy.

Methodology: *In vitro* experimental study was conducted in the Mycology Laboratory at Dermatovenereology Outpatient Clinic Dr. Soetomo General Hospital Surabaya. Eighteen isolates of *Candida* species were isolated from the vaginal thrush of 15 pregnant women diagnosed with VVC from March to May 2021. Antifungal susceptibility of TTO 5% and TTO 10% was evaluated by the disc diffusion method, with the inhibitory zone diameter as the main outcome.

Results: The mean inhibitory zone diameter of TTO 5%, TTO 10%, and nystatin against all *Candida* spp. was 7.26 mm, 8.64 mm, and 25.57 mm, respectively ($p < 0.001$). The mean inhibitory zone diameter of TTO 5%, TTO 10%, and nystatin tend to be larger in *C. albicans* compared to the non-*albicans*, but the difference is not significant. Nystatin displayed the largest mean inhibitory zone diameters compared to TTO 5% and TTO 10% ($p < 0.001$) in all *Candida* species. Increased concentration from TTO 5% to TTO 10% resulted in a slight increment in the mean inhibitory zone diameters in all-*Candida* species ($p = 0.001$).

Conclusions: *Tea Tree Oil* displayed antifungal activity against *Candida* species causing VVC in pregnancy. Further studies are required to investigate optimal TTO concentrations as a VVC treatment in pregnancy.

Key words: Vulvovaginal candidiasis; *tea tree oil*; nystatin; inhibition zone; sexual and reproductive health care

J Infect Dev Ctries 0000; 0(00):000-000. doi:10.3855/jidc.16761

(Received 30 April 2022 – Accepted 16 January 2023)

Introduction

One of the most common gynecological problems is vulvovaginal candidiasis (VVC), and in pregnant women, the incidence of VVC is almost doubled [1]. Candidiasis in pregnancy often results in a less effective response to treatment [2]. The incidence of VVC in Dr. Soetomo General Academic Teaching Hospital was still relatively high, with 16 (53%) of the 30 smear samples collected from the posterior vaginal fornix of pregnant women testing positive for *Candida* species [3]. Complications of VVC in pregnancy have been linked to an increased risk of prematurity and low birth weight and significant discomfort to the mother, including redness, increased discharge, burning sensation in the vulva area, and itching [1,4].

According to epidemiological and clinical statistics, topical therapy does not effectively remove *Candida* infection in the vaginal environment, and about 13% of patients develop recurrent VVC that develop into chronic infections [5]. As many as 5 of 16 women will relapse. Due to the uncomfortable nature, most women employ natural products or drugs that are not licensed for treatment or relapse prevention [6,7].

Essential oils from some plants, such as *Melaleuca alternifolia*, have been studied for their *in vitro* microbicidal actions and their ability to decontaminate the vaginal canal and prevent fungal colonization by

combining fungicidal action [6]. *Tea tree oil* (TTO) significantly inhibits de novo biofilm formation. In many countries, TTO-containing medications are used to treat vaginal infections and are specially manufactured to be administered intravaginally [8,9]. However, there were no studies on the effectiveness of TTO in treating VVC during pregnancy in Indonesia. Therefore, it is crucial to develop new antifungal agents that broaden their spectrum against *Candida* spp. and become an alternative combination option between conventional and standard antifungal agents in the treatment of VVC during pregnancy. This study aims to assess the antifungal activity of TTO 5%, and TTO 10% compared with nystatin against *Candida* spp. causing VVC in pregnancy.

Methodology

Subjects

This study was carried out *in vitro*. The study design has been approved by Ethics Committee of Dr. Soetomo General Academic Teaching Hospital Surabaya. The study protocol was explained to subjects wishing to participate in this study by providing study clarification. Patients gave their consent prior to the study. Samples were collected during a 3-month period between March 2021 to May 2021 using total sampling method, and 15 pregnant patients attending Obstetrics and Gynecology outpatient clinic were diagnosed with VVC. Inclusion criteria of subjects were patients diagnosed with VVC having at least one of the VVC symptoms (vaginal discharge like cheese or cracked milk, vaginal itching, erythema, dyspareunia) and fungal positivity (*pseudohyphae* and/or *blastospores*) by Gram staining smears. VVC pregnant patients treated with antifungal therapy, intravaginally or systemically, within the last 2 weeks and with no colony growth on *CHROMagar* media were excluded from the study.

Clinical isolate

Two swabs were taken simultaneously from each patient and immediately sent to Mycology Laboratory at the Clinical Microbiology Laboratory of Dr. Soetomo General Hospital Surabaya. The 1st swab was used to make thin smears on microscopic slides for Gram staining and the second swab was cultured on *CHROMagar* media. The cultures were cultivated for 36-48 hours before species identification. In this study, 18 isolates of *C. albicans* species and non-*albicans* were found. The susceptibility of antifungal was assessed by the disc diffusion method on *Mueller Hinton* agar with 2% glucose and methylene blue. *Candida* spp. isolates were plated on agar and paper discs containing TTO 5%, TTO 10%, and nystatin were placed on the media. The cultures were incubated for as long as 24-48 hours, and the inhibition zone was measured with a Vernier caliper. The diameter of zones of inhibition between TTO 5%, TTO 10%, and nystatin were then compared.

Results

A total of fifteen (15) subjects were assigned for isolation and identification of *Candida* species and antifungal susceptibility test. Subjects' characteristics are presented in Table 1. The majority of subjects were in the 3rd trimester of pregnancy (11; 73.3%), followed by the 2nd trimester of pregnancy (4; 26.7%). No subject was in the 1st trimester of pregnancy as shown in Figure 1.

Among the 18 vaginal isolates collected from pregnant women with vulvovaginal candidiasis (VVC), four *Candida* species were isolated and identified: *Candida albicans*, *Candida glabrata*, *Candida dubliniensis*, and *Candida parapsilosis.* Seven (38.8%) of the isolates were *C. albicans* while eleven (11) isolates were non-*albicans* (61.1%) species, consisting of *C. glabrata* (45.4%), *C. dubliniensis* (36.3%) and *C.*

Table 1. Demographic data of subjects.

No.	Basic Data			
	Category	**Group**	**N**	**%**
1	Age	Late teens (18 – 25 year)	2	13.3
		Early adult (26 – 35 year)	12	80
		Late adult (46 – 55 year)	1	6.7
		Early senior adult (46 – 55 year)	0	0
		Late senior adult (56 – 65 year)	0	0
2	Education	Elementary school	0	0
		Junior high school	3	20
		High school	5	33.3
		Diploma/bachelor	7	46.7
3	Occupation	Housewives	10	66.7
		Private sector employee	2	13.3
		Government employee	1	6.7
		Nurse	2	13.3

Figure 1. The distribution of patients' gestational age.

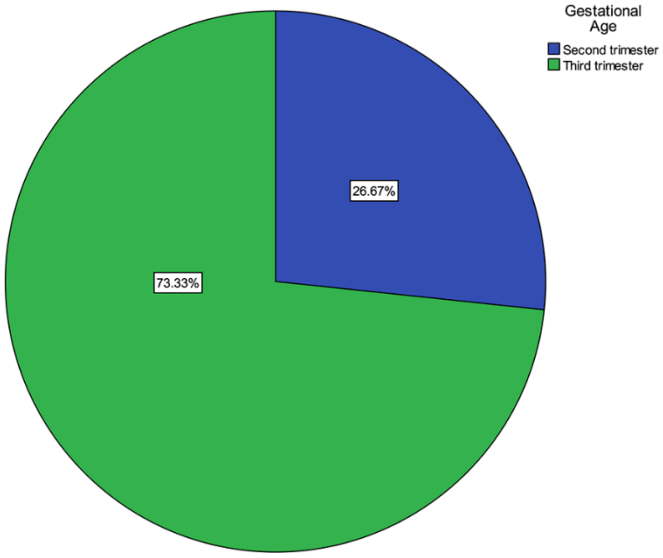
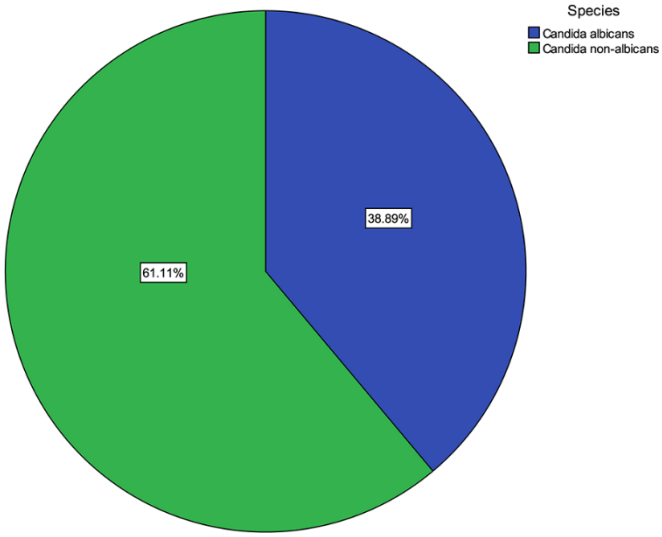


Figure 2. The distribution Candida species found in patients.



parapsilosis (18.1%) as shown in Figure 2 and Figure 3. More than one species of *Candida* was found in 3 subjects.

All *Candida* species showed growth inhibition in TTO 5%, TTO 10%, and nystatin. The mean inhibitory zone diameter of TTO 5% in all isolates was 7.26 mm, while *C. albicans* and non-*albicans* had 7.77 mm and 6.85 mm mean inhibitory zone diameters, respectively. The mean inhibitory zone diameter of TTO 10% in all isolates was 8.64 mm, while *C. albicans* and non-*albicans* had 9.01 mm and 8.36 mm mean inhibitory zone diameters, respectively. Compared to TTO 5%, TTO 10% displayed slightly larger mean inhibitory zone diameters in all-*Candida* species but was statistically significant ($p = 0.001$). However, we could not determine TTO sensitivity because there were no Clinical and Laboratory Standards Institutes (CLSI) criteria for TTO.

The mean inhibitory zone diameter of nystatin in all isolates was 25.57 mm, while the mean inhibitory zone diameter in *C. albicans* and non-*albicans* was 25.94 mm and 25.29 mm, respectively. Sensitivity criteria according to CLSI for nystatin were used to determine *Candida* sensitivity (Table 2). According to the criteria, all isolates were sensitive to nystatin, and no strains were resistant to it.

In all Candida species, the mean diameter of the nystatin inhibition zone was larger than TTO 5% and TTO 10%, which was 25.57 mm compared to 7.26 mm and 8.64 mm, respectively (Figure 4). Kruskal-Wallis tests revealed a significant difference between the diameters of the inhibitory zone between TTO 5%, TTO 10%, and nystatin ($p < 0.001$), whereby nystatin was superior to both TTO 5% and TTO 10% in both *C. albicans* and non-*albicans*. In this study, we found that the mean inhibitory zone diameters of TTO 5%, TTO 10%, and nystatin was slightly larger in *C. albicans*

Figure 3. The distribution of Candida non-albicans species found in patients.

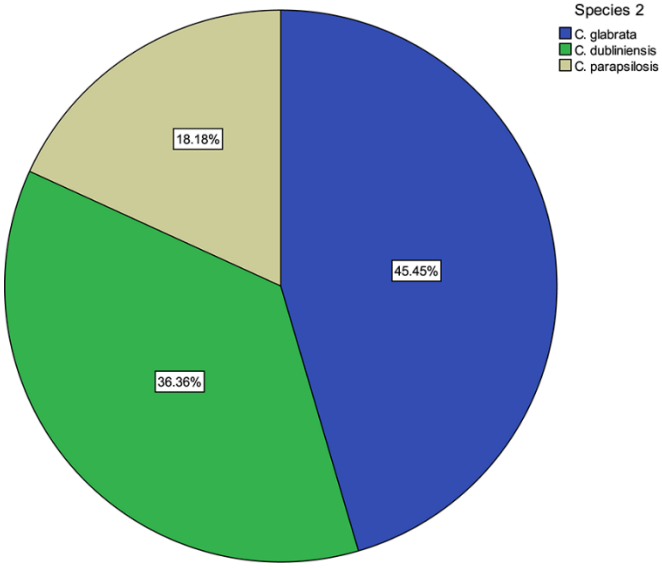


Table 2. Nystatin inhibition zone for Candida species (10).

Drug	Concentration	Inhibition Zone Diameter (mm)		
		Sensitive	Intermediate	Resistant
Nystatin	100 U/disc	≥ 15	10-14	≤ 10

compared to *C.* non-*albicans*, but the difference was not statistically significant (Figure 5).

Discussion

According to previous reports, one-quarter of all women are exposed to vaginal candidiasis during their lives [11]. This study revealed a total of 15 pregnant women with vulvovaginal candidiasis (VVC) during three months period between March 2021 and May 2021 at Dr. Soetomo General Hospital. The prevalence of VVC in this study was at a higher frequency within the age range 26-35 years (80%) because women in this age range are younger and sexually active. Moreover, some women in this age range are becoming more desirous of having children. They also have a possible low vaginal defense mechanism against *Candida* species and have increased levels of estrogen and corticoids and, therefore, are more susceptible to *Candida* species infections [11]. This study found that the women in the 3rd trimester had the highest prevalence rate of VVC. In 3rd trimester pregnant women, symptomatic recurrences are more likely, and therapeutic response is diminished [12]. The immune system of pregnant women in the 3rd trimester of pregnancy is weakened compared to the 2nd and 1st trimesters, increasing the chance of *Candida* species becoming pathogenic. Vaginal colonization and symptomatic vaginitis are more common during pregnancy. These factors contributed to the highest prevalence of VVC in the 3rd trimester of pregnancy.

Figure 4. The mean inhibitory zone diameters of TTO 5%, TTO 10%, and nystatin in all Candida species.

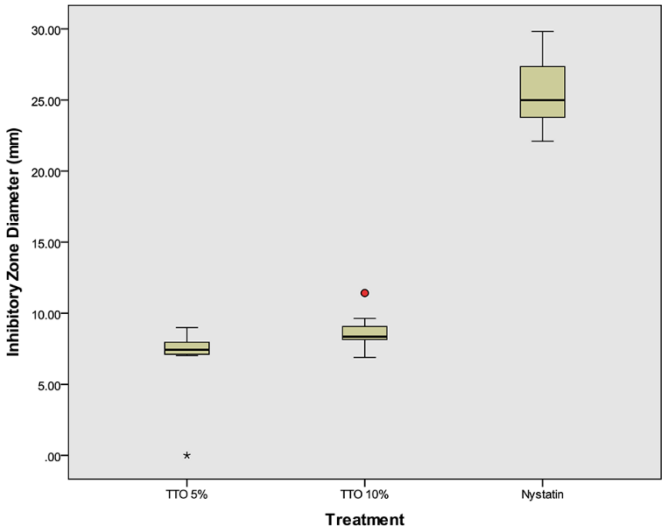
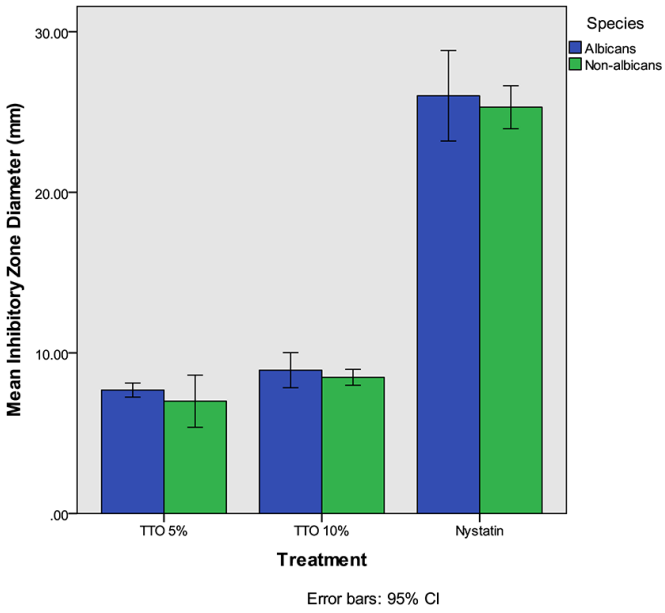


Figure 5. The comparison between mean inhibitory zone diameters of TTO 5%, TTO 10%, and nystatin in C. albicans and non-albicans.



Candida albicans is the most common cause of fungal infections of the reproductive tract in women of childbearing age [13]. However, there have been reports in recent years of *Candida* non-*albicans* species being identified from VVC patients, especially *Candida glabrata*, which is increasingly being identified as the infection's source [14,15]. Our findings showed that the most common *Candida* species isolated from vaginal discharge were non-*albicans* there are *C. glabrata* (54.4%), followed by *C. dubliniensis* (36.3%), and *C. parapsilosis* (18.1%). A similar study by Nelson *et al.* reported that *C. glabrata* was the most common cause of VVC in pregnant women [16]. The shift towards *C.* non-*albicans* as the cause of VVC is concerning for pregnant women, as it has the potential to make VVC chronic, recurrent, and more resistant to antifungal drugs than *C. albicans*. The high resistance levels of *C.* non-*albicans* species to routinely used medications, together with an increase in their identification in women with VVC, emphasizes the need to identify *Candida* species in vaginal samples to give clinicians information about the best treatment for their patients.

Tea tree oil (TTO) at a concentration of up to 20% was considered safe and without major adverse effects [17]. This study used TTO with 5% and 10% concentrations. This concentration was chosen to prepare the medication for future human use by using the minimal concentration that was effective with low toxicity risk. TTO 5% and TTO 10% showed antifungal activity against *Candida* species in this study. This effect was proved by the TTO inhibition produced in the media with a diameter of 7.26 mm and 8.64 mm in

all *Candida* species. According to Hammer *et al.*, TTO and their components improved membrane cell permeability, and this could inhibit the growth of *Candida* [18]. *Tea tree oil* could also produce membrane lipid bilayers that change the component keeping the membrane intact. These changes lead to the inhibition of the growth of *Candida* [19]. The mean diameter of TTO inhibition zone of *C.* non-*albicans* was slightly lower than *albicans* species meaning that the antifungal susceptibility of *C. albicans* was better than those of *C.* non-*albicans.*

Non-*albicans* candidiasis is more likely among those with diabetes mellitus, the elderly, prior antifungal drugs, or low socioeconomic status. The pathogenic mechanisms of *C.* non-*albicans* are less well understood than those of *C. albicans*, where more extensive research has been carried out [20]. Intrinsic resistance or low-dose susceptibility to azole antifungals, the first-line treatment, is a prominent feature of non-*albicans* species, which leads to treatment failure [21]. Identification and antifungal susceptibility testing are required for optimal treatment in pregnant women of these infections, especially in settings where the diagnosis is based on clinical presentation or limited laboratory testing.

The inhibitory zone of nystatin was significantly higher than TTO in diameter. This could be due to different drug mechanisms. Nystatin was discovered to bind ergosterol, which is the primary component of the fungal cell membrane. It created a pore-like structure, which allowed plasma to flow out, resulting in fungal cell death [22]. This was different from the TTO mechanism, which increased the permeability of the fungal cell membrane but did not form a pore-like structure as in nystatin. *Tea tree oil* was fungistatic, but only at higher concentrations did it become fungicidal [23]. *Tea tree oil* has also been shown to inhibit biofilm formation. The ability of *C. albicans* to adhere to and form a biofilm is very important in the incidence of VVC. The antifungal activity of TTO and *terpinen-4-ol* was able to control the proliferation of biofilms *in vitro* [24,25]. On the other hand, nystatin lacks this mechanism. Therefore, TTO can be considered as adjuvant therapy for VVC in pregnancy.

In both *C. albicans* and *C.* non-*albicans*, there was a statistically significant difference in the inhibitory zone diameters of TTO 5% and TTO 10% compared to nystatin ($p < 0.001$). The inhibitory zone diameters of *C. albicans* were slightly larger than other *Candida* species but were not statistically different. Based on this result, further study of TTO antifungal activity was needed to establish the optimal concentration of antifungal treatment of VVC in pregnancy. *Tea tree oil* must be used in higher concentrations to prevent *C. albicans* from growing [26]. This could be due to the ability of *C. albicans* to form a germination tube and biofilm, which would protect the fungi from TTO-induced environmental changes [27]. *Tea tree oil* demonstrated fungistatic effects, but only at higher concentrations did it have a fungicidal effect (26). In our study, we found a significant difference in the inhibition zone diameter between TTO 5% and TTO 10% for each species of *C. albicans* and *C.* non-*albicans*, but the increment in antifungal activities with increasing TTO concentrations were minimum. Given the risks of administering TTO, especially during pregnancy, a concentration of TTO 5% may be sufficient to offer a therapeutic effect on *Candida* that causes VVC.

According to this study, our opinion is that combining TTO with standard medications such as nystatin might be helpful to treat chronic VVC or VVC in pregnancy. Further studies are required to determine the antifungal activity of natural medicinal components and to uncover synergistic interactions with routinely used antifungal drugs.

Acknowledgements

The authors would like to thank the Faculty of Medicine, Universitas Airlangga for research funding under the scheme of Penelitian Unggulan Fakultas with the reference number 212/UN3/2021.

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Conflict of interests: No conflict of interests is declared.