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OPEN PEER REVIEW

# Phytochemical Composition, Antibacterial, and Antibiofilm Activity of *Malva sylvestris* Against Human Pathogenic Bacteria

Mahmoud Fathi, Maryam Ghane, Leila Pishkar

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## About this report

This open peer review (OPR) report shows the public comments made on this article during peer review, by the reviewers, the associate editor and the editor-in-chief, for every revision round, together with the authors' replies. Comments appear only with the consent of the people who wrote them. Reviewers who chose to sign their report are named; the others are shown as "Referee". Comments are edited for language only, and confidential remarks to the editors are not included.

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## Contents

1 **Original submission** Olusegun Abayomi Olalere, Pardis Mohammadi Pour, Hossein Motamedi, Assoc... 2

2 **Revision 1** Olusegun Abayomi Olalere, Referee 1, Associate Editor, Editor-in-Chief 6

**Authors' reply to reviewers** 7

**Reviewer and editor comments** 15

3 **Revision 2** Referee 1, Associate Editor, Editor-in-Chief 16

**Authors' reply to reviewers** 17

**Reviewer and editor comments** 19

## 1 Original submission

5 comments · 9 Apr 2021 – 3 May 2021

### Olusegun Abayomi Olalere Reviewer

MAJOR REVISION 9 Apr 2021

Abstract
- The background of the study is too scanty. Add a few lines to clearly state the problem addressed in your study.
-Rephrase "...."
-Your results indicate that apigenin and salicylic acid were identified using HPLC. What does the bioavailability of these two components indicate about the biological activity of your sample?
-Rephrase your conclusion by relating it to the obtained results.

Keywords: add two more keywords

Introduction:
- The research gap is missing.
-Give a concise review of previous literature on other analyses conducted on this same sample.
State the limitations of previous studies and the new knowledge in your current studies.
-Your references are not recent. Include recent studies, i.e., 2020-2021.

Methods:
-Add the country of manufacture of all the equipment used, e.g., the rotary evaporator, etc.
-Check your units and make sure you are consistent with SI units.
-Your equations should be reformatted using the equation editor, e.g., in section 2.5.

Results:
-Please compare your results with previous studies.
-This discussion is not concise. Try to rewrite it.

Conclusion:
Add more to the conclusion and give an overall overview of the results and their implications.

### Pardis Mohammadi Pour Reviewer

MAJOR REVISION 28 Apr 2021

Dear Author
The manuscript represents valuable results. The study design and methods are perfect. Please consider the following comments.
Check the grammar once more. There are grammatical errors.
All the scientific names should be in italics.
Abstract Background: Medicinal plants have been considered as antimicrobial agents since thousands years ago and using medicinal plants hasn't been dedicated to recent years.
Abstract conclusion: Why do the antibacterial and antibiofilm activities of M. sylvestris reported in the present study lead the authors to suggest the use of these herbal compounds as alternatives to antimicrobial drugs?
These results are not in the range of effective concentrations of the extract in biological studies. What is the reference for your measurement to support using these results as alternatives to antimicrobial drugs?
Method: Please provide details about Gas Chromatography and mass spectroscopy, including the temperature, instrument characteristics, and the kind of library used.

In Phenolic compounds analyses, it's not clear how the 18 compounds were identified. If just 2 of the compounds were identified by HPLC, how were the other compounds identified? Also, please explain more about the solvent system (isocratic or gradient), the kind of solvent, whether the column was normal-phase or reverse-phase, and the brand of the HPLC column.

**Hossein Motamedi** Reviewer

MAJOR REVISION 3 May 2021

Dear author
Please find the comments as follows:
1- With regard to structural differences between the cell walls of gram-positive and gram-negative bacteria, how can the differences between the results obtained against the test bacteria be interpreted?
2- The cell wall of S. aureus is very resistant due to the high degree of transpeptidation linkage between the side chains of peptidoglycan. So how can the results obtained against this bacterium be compared with those obtained against other gram-positive as well as gram-negative bacteria?
3- It is necessary to provide a serious discussion of the antibacterial mechanism of the active constituents found in the extracts.
4- The manuscript needs serious language editing.
5- It is necessary to compare the active components of other extracts of M. sylvestris that have been mentioned in the discussion with those of the methanolic extract that has been tested in this study, for example, the ethanolic extract and so on.
6- Some comments have been noticed inside the manuscript.

**Associate Editor**

MAJOR REVISION 3 May 2021

Dear Dr. Ghane:

The manuscript entitled "Phytochemical composition, antibacterial, and antibiofilm activity of Malva sylvestris against human pathogenic bacteria", which you submitted to the JJNPP, has been reviewed. The comments of the reviewer(s) are included at the bottom of this letter.
The reviewer(s) have recommended major revisions to your manuscript. Therefore, I invite you to respond to the reviewer(s)' comments and revise your manuscript.
Reviewer1:
The manuscript represents valuable results. The study design and methods are perfect. Please consider the following comments.
1-Once more, check the grammar. There are grammatical errors.
2-All the scientific names should be italicized.
3-Abstract Background: Medicinal plants have been considered antimicrobial agents for thousands of years, and their use hasn't been limited to recent years.
4-Abstract conclusion: Why do the antibacterial and antibiofilm activities of M. sylvestris reported in the present study lead the authors to suggest the use of these herbal compounds as alternatives to antimicrobial drugs?
These results are not within the range of effective concentrations of the extract in biological studies. What is the reference for your measurement that supports using these results to suggest alternatives to antimicrobial drugs?
5-Method: Please explain the temperature, instrument characteristics, and type of library used for Gas Chromatography and mass spectroscopy.

6-In the phenolic compound analyses, it's not clear how the 18 compounds were identified. If just 2 of the compounds were identified by HPLC, how were the other compounds identified? Also, please explain more about the solvent system (isocratic or gradient), type of solvent, whether the column was normal-phase or reversed-phase, and the brand of the HPLC column.
Reviewer2:
Abstract
1- The background of the study is too scanty. Add a few lines to clearly state the problem addressed in your study.
2-Rephrase "...."
3-Your results indicate that apigenin and salicylic acid were identified using HPLC. What does the bioavailability of these two components indicate about the biological activity of your sample?
4-Rephrase your conclusion by relating it to the obtained results.
5-Keywords: Add two more keywords.
Introduction:
6- The research gap is missing.
7-Give a concise overview of previous literature on other analyses conducted on this same sample.
State the limitations of previous studies and the new knowledge in your current studies.
8-Your references are not recent. Include recent studies, i.e., 2020-2021.
Methods:
9-Add the country of manufacture of all the equipment used, e.g., the rotary evaporator.
10-Check your units; make sure you are consistent with SI units.
11-Your equations should be reformatted using Equation Editor, e.g., in section 2.5.
Results:
12-Please compare your results with previous studies.
13-This discussion is not concise. Try to rewrite it.
Conclusion:
14-Add more to the conclusion and give an overall overview of the results and what they indicate.
Reviewer3:
Please find the comments as follows:
1- With regard to structural differences between the cell walls of gram-positive and gram-negative bacteria, how can the differences between the results obtained against the test bacteria be interpreted?
2- The cell wall of S. aureus is very resistant due to its high transpeptidation linkage between side chains of peptidoglycan. So how can the results obtained against this bacterium be compared with those obtained against other gram-positive as well as gram-negative bacteria?
3- It is necessary to discuss the antibacterial mechanism of active constituents found in the extracts in depth.
4- The manuscript needs serious language editing.
5- It is necessary to compare the active components of other extracts of M. sylvestris that have been mentioned in the discussion with those of the methanolic extract that has been tested in this study, for example, the ethanolic extract and so on.
6- Some comments have been noted inside the manuscript.

With regards
Neda Bavarsad

**Editor-in-Chief** MAJOR REVISION 3 May 2021

Dear Authors,

We have received the reviewers' comments on your manuscript; they suggest a major revision.

Please revise your manuscript accordingly, and send your revised manuscript, including a point-by-point response to the comments. We will reevaluate it as soon as we receive it.

Kind REgards,
JJNPP
Editor-in-Chief

2

# Revision 1

AUTHORS' RESPONSE TO REVIEWERS

Authors' reply to reviewers

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

**To: Editor-in-Chief of**: **Jundishapur Journal of Natural Pharmaceutical Products**

**Dear Professor Eskandar Moghimipour**

Thank you very much for giving us the opportunity to improve the manuscript entitled
**“**Phytochemical composition, antibacterial, and antibiofilm activity of *Malva sylvestris* against human pathogenic bacteria**”.**

Manuscript has been revised according to the reviewer’s suggestions. The individual issues raised by the reviewers are listed below together with our actions and comments. All the changes are highlighted in the text.

**Reviewers' Comments:**

Reviewer 1:
Dear Author
The manuscript represent valuable results. The study design and methods are perfect. Please consider the following comment.

1-Once more check the grammar. There are grammatical errors.

Action/comment:

The grammar was checked and revised.

2- All the scientific names should be in italic form.

Action/comment:

The scientific names were written in italic form as suggested.

3- Abstract Background: Medicinal plants have been considered as antimicrobial agents since thousands years ago and using medicinal plants hasn’t been dedicated to recent years.

Action/comment:

Abstract: Background was rewritten.

4-Abstract conclusion: why does the Antibacterial and antibiofilm activity of *M. sylvestris* reported in the present Study cause that authors suggest the use of these herbal compounds as alternatives to antimicrobial drugs? These results are not in the range of the effective concentrations of the extract on biological studies. What is the reference of your measurement to use these result as alternatives to antimicrobial drugs?

Action/comment:

Since the *M. sylvestris* extract represented appreciable antibiofilm activity, we suggest it as alternative antimicrobial drug. Of course, further *in vitro* and *in vivo* studies are needed for this claim. To clear up this ambiguity, we rewrote the conclusion.

5-Method please explain about Gas Chromatography and mass spectroscopy the temperature, instrument characteristics the kind of library which was used.

Action/comment:

Gas Chromatography was explained as reviewer suggested.

6-In Phenolic compounds analyses it’s not clear how the 18 compounds were identified? If just 2 of the compounds were identified by HPLC so how the other compounds were identified? Also please explain more about the solvent system (isocratic or gradient), kind of solvent , normal or revers column the brand of the HPLC column

Action/comment:

In phenolic compounds analyses, HPLC analysis was performed to quantify the amount of eighteen phenolics of which, only two compounds apigenin and salicylic acid were identified. It means that among eighteen phenolics only two components were detected and the other 16 compounds were not identified. We stated it in discussion.

We explained about HPLC as suggested by reviewer in method section.

Reviewer 2:

Abstract
1- Background of study is too scanty. Add few lines to clearly state the statement of problem of your study.

Action/comment:

Background of study was rewritten and few lines were added as the reviewer suggested.

2-Rephrase "...."

3 -From your results apigenin and salicylic acid was identified using HPLC, what's the bioavailability indication of these two components on the biological activity of your sample.

Action/comment:

We did not assess the antimicrobial activity of apigenin and salicylic acid against tested bacteria. However, numerous studies (please see below) reported that these two compounds have antimicrobial activity and the antimicrobial activity of *M. sylvestris* maybe due to the presence of these compounds. These are stated in the text (in discussion section).

Nayaka HB, Londonkar RL, Umesh MK, Tukappa A. Antibacterial attributes of apigenin, isolated from Portulaca oleracea L. International journal of bacteriology. 2014;2014.

Kantouch A, El-Sayed AA, Salama M, Abou El-Kheir A, Mowafi S. Salicylic acid and some of its derivatives as antibacterial agents for viscose fabric. Int J Biol Macromol. 2013;62:603-7.

4-Rephrase your conclusion by relating it with the obtained results.
Action/comment:

The conclusion was rewritten as suggested by reviewer.

5-Keywords: add two more key words

Action/comment:

Two more key words were added.

Introduction:
6- Research gap is missing.

Action/comment:

Research gap was stated in the introduction section (we stated it as limitation of previous studies).

7- Give a concise past literature on other analysis conducted on this same sample. State the limitations of previous studies and the new knowledge in your current studies.

Action/comment:

The introduction was improved according to the reviewer suggestion and the limitation of previous studies was stated.

8-Your references are not recent. Include recent studies i.e 2020-2021.

Action/comment:

Four new references (recent studies i.e 2020-2021) were added. (9, 34, 13, 24).

Methods:
9-Add the country of manufacture of all the equipment used e.g rotary evaporator etc.

Action/comment:

The country of manufacture of all the equipment was added.

10-Check your units, make sure you are consistent with SI units...

Action/comment:

The units were checked.

11-Your equations should be reformatted using equation Editor. e.g in section 2.5.

Action/comment:

The equations were reformatted using equation Editor in section 2.5.

Results:
12-Please compare your results with previous studies.

Action/comment:
We compared the results with previous studies.

13-This discussion is not concise...Try and rewrite.

Action/comment:

The discussion was rewritten.

Conclusion:
14- Add more to the conclusion and give the overall overview of the results and the indication of the results.

Action/comment:
Conclusion was rewritten.

Reviewer 3:
Dear author
Please find the comments as follow:

1- With regard to structural differences between cell wall of gram positive and gram negative bacteria, how can it be interpreted the differences between the results obtained against test bacteria?

Action/comment:

In Gram-positive bacteria peptidoglycan layers are porous and allow antibiotics, chemical compounds and even herbal drugs to access the cell membrane. While

the lipopolysaccharides layer and periplasmic space of Gram-negative bacteria may be the reason for their relative resistance to antimicrobials.
This is also stated in discussion section.

2- The cell wall of *S. aureus* is very resistant due to its high transpeptidation linkage between side chains of peptidoglycan. So how it can be compared the results obtained against this bacterium in comparison to other gram positive as well as gram negative bacteria?

Action/comment:

Although there are high transpeptidation linkage between side chains of peptidoglycan in *S. aureus*, peptidoglycan is relatively *porous*, and most substances can pass through the Gram-positive cell wall with little difficulty.

3- It is necessary to do serious discussion about the antibacterial mechanism of active constituents found in extracts.

Action/comment:
The discussion was improved and the reason for susceptibility of Gram-positive bacteria to extract was stated.

4- The manuscript needs serious language editing.

Action/comment:
It was edited.

5- It is necessary to compare active components of other extracts of *M. sylvestris* that have been mentioned in discussion with the methanolic extract that has been tested in this study. For example the ethanolic extract and so on.

Action/comment:
Active compounds obtained in this study was compared with other studies in discussion section.

6- Some comments have been noticed inside the manuscript.

Action/comment:
The text revised according to the comments.

Except:

Routinely the plant extracts are dissolved in the relevant solved used for their extraction. Why you used water and DMSO instead of Methanol?

Action/comment:
In a number of studies dry extracts has been prepared by dissolving the extract in DMSO. This can be observed in:

1-Shadid KA, Shakya AK, Naik RR, Jaradat N, Farah HS, Shalan N, et al. Phenolic Content and Antioxidant and Antimicrobial Activities of Malva sylvestris L., Malva oxyloba Boiss., Malva parviflora L., and Malva aegyptia L. Leaves Extract. Journal of Chemistry. 2021;2021.

2-Njeru SN, Obonyo MA, Nyambati SO, Ngari SM. Antimicrobial and cytotoxicity properties of the crude extracts and fractions of Premna resinosa (Hochst.) Schauer (Compositae): Kenyan traditional medicinal plant. BMC Complement Altern Med. 2015;15(1):1-9.

Best redards

## 2 Revision 1

4 comments · 25 May 2021 – 25 May 2021

**Olusegun Abayomi Olalere** Reviewer — ACCEPT — 25 May 2021

The author has sufficiently amended the manuscript according to my last comment.

**Referee 1** Reviewer — MINOR REVISION — 25 May 2021

Dear author;

Thank you for the correction and modifications. But there is still one point:

I think you did not understand Comment 5 from reviewer 3:
It is necessary to compare the active components of other extracts of M. sylvestris that have been mentioned in the discussion with those of the methanolic extract that has been tested in this study. For example, the ethanolic extract and so on.

Your answer:
Action/comment: Active compounds obtained in this study were compared with other studies in discussion section.

Please add at least one comparison between the active components of other kinds of extracts (e.g. ethanolic) of the plant and those of the methanolic extract (that you worked on).

good Luck!

**Associate Editor** — MINOR REVISION — 25 May 2021

Dear Dr. ghane
It is necessary to compare the active components of other extracts of M. sylvestris that have been mentioned in the discussion with those of the methanolic extract tested in this study. Examples include the ethanolic extract and so on.

**Editor-in-Chief** — MINOR REVISION — 25 May 2021

Dear Authors,

Your revised manuscript still needs a minor revision. We will consider it for further processing as soon as you submit your revised version. Please remember to include a point-by-point response to the comments.

JJNPP EIC

3

# Revision 2

AUTHORS' RESPONSE TO REVIEWERS

Authors' reply to reviewers

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

**To: Editor-in-Chief of**: **Jundishapur Journal of Natural Pharmaceutical Products**

**Dear Professor Eskandar Moghimipour**

Thank you very much for giving us the opportunity to improve the manuscript entitled

**"**Phytochemical composition, antibacterial, and antibiofilm activity of *Malva sylvestris* against human pathogenic bacteria**".**

Manuscript has been revised according to the reviewer's suggestion. The issue raised by the reviewer is listed below together with our action and comment. All the changes are highlighted in the text.

I think you did not get the Comment 5 from reviewer 3;
It is necessary to compare active components of other extracts of M. sylvestris that have been mentioned in discussion with the methanolic extract that has been tested in this study. For example the ethanolic extract and so on.
Please add at least one comparison between active components of another kinds of extracts (e.g. ethanolic) of the plant and methanolic extract (that you worked on).

**Action/Comment**

We added comparison between compounds of another kind of extract of *M. sylvestris* and methanolic extract that we worked on.

In GC analysis we add two studies about methanolic and hydroalcoholic extract of *M. sylvestris.*

We also added two studies about active components obtained by HPLC from methanolic and **ethanolic** extract of *M. sylvestris*.

Best Wishes

## 3 Revision 2

3 comments · 12 Jun 2021 – 13 Jun 2021

### Referee 1 Reviewer

ACCEPT 12 Jun 2021

Dear authors;
Thank you for all the considerations!
It is acceptable now.

Good Luck!

### Associate Editor

ACCEPT 12 Jun 2021

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

### Editor-in-Chief

ACCEPT 13 Jun 2021

Dear Authors,

I am glad to inform you that your manuscript has been accepted for publication in its current form; it will be sent to the publisher for further processing.
Thank you for your interest in our journal.

Kind Regards,
JJNPPEIC