



NATIONAL INSTITUTE FOR BIOTECHNOLOGY AND GENETIC ENGINEERING (NIBGE),

PO BOX 577, Jhang Road, Faisalabad, Pakistan. +92 41 2651471, www.nibge.org



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Dated: 09-06-2020

RD -1: FORM FOR SUBMISSION OF RESEARCH PAPER /PRESENTATION

Title of paper:	Screening and identification of bioactive compounds in citrus fruit extracts against Hepatitis C Virus NS3 protease (genotype 3a) using Fluorescence Resonance Energy Transfer assay and Mass Spectrometric techniques.			
Type of Paper (Tick appropriate column)	Research Paper ✓	Review Article	Note/Communication	Presentation
Name of Journal/ Conference/Meeting	World Journal of Gastroenterology			

Fill in the names of authors in order of their relative communications:

Name of author (position held)	Contribution (Tick whichever is applicable)				Overall % Contribution	Signature of Authors
	Basic Idea	Expt/Theor y	Data Analysis	Writing of Manuscript		
Mahim Khan	✓	✓	✓	✓	40 %	(Mahim Khan)
Waqar Rauf			✓		10 %	(Waqar Rauf)
Moazur Rahman		✓	✓		10 %	(Moazur Rahman)
Fazal-e-Habib		✓		✓	10 %	(Fazal-e-Habib)
Mazhar Iqbal	✓	✓	✓	✓	30 %	(Mazhar Iqbal)

ABSTRACT/ SUMMARY

Background: Hepatitis C Virus Genotype 3a is highly prevalent in Pakistan. Due to the elevated costs of available FDA approved drugs against HCV, medicinal natural products having antiviral potential could be screened for the cost-effective prevention of the disease. Using such products, inhibition assays against vital viral proteins like NS3 protease could be developed to prevent viral proliferation in host.

Aims: Efforts have been made to get highly purified and functionally active NS3 protease in *E. coli* expression system. The protease activity was tested for inhibition in the presence of flavonoid rich citrus fruit extracts using Fluorescence Resonance Energy Transfer (FRET) assay. Potent extracts was further analyzed using LCMS/MS technique to identify active compounds against the protease.

Methods: The codon optimized NS3 protease domain alone and in fusion with its co-factor NS4a protease was expressed in *E. coli* BL21 (DE3) using pET11a expression vector. The expressed protein was purified using metal ion affinity chromatography followed by polishing using gel filtration. After that, citrus fruit extracts were screened using FRET assay against the protein and polyphenols were identified as potential inhibitors using ESI-MS/MS technique. Among different polyphenols, highly potent compounds were screened using Docking and the active compound was further evaluated against NS4A-NS3 protease using FRET.

Results: NS4a fused NS3 gene was overexpressed and purified protein yield was high in comparison to the lower yield of the full length NS3 protein. The native purified NS4-NS3 fused protein was tested against citrus extract and grapefruit mesocarp extract showed lowest percentage inhibition value of 0.0004642 %. Hesperidin was the active compound in the extract having docking S-score value -10.9818.

Certified that this study was approved for Bio Safety Level, Environmental and Social Safeguard

Sr. No.	Authors	Field of specialization	Area of input
1	Mahim Khan, PhD scholar, HBD	Protein Chemistry/ LCMS/MS data analysis	She is working as a PhD student in this project. She has optimized the expression and purification of HCV NS3 protease. She has also extracted antioxidant compounds and tested their inhibition studies using <i>invitro</i> FRET assay. Furthermore, she has performed LCMS/MS technique to identify polyphenols in plant extracts and check their interaction studies using modelling approach. She was involved in this project from designing to execution and writing this manuscript. She has done part of this work during her IRSIP Fellowship at National Institute of Health, USA.
2	Waqar Rauf, SS, HBD	LCMS/MS data analysis	He has contributed in designing and execution of the experiments and data analysis.
3	Moazur Rahman, PS, HBD	Protein Chemistry	He has provided help in HCV NS3 protease and is also serving as a PhD Co-supervisor of Ms. Mahim Khan.
4	Fazal-e-Habib, SA-I, HBD	LCMS/MS running / operation	He is the main person who is operating LCMS/MS on day to day basis (at NIBGE).
5	Mazhar Iqbal, Supervisor & PS, HBD	Bioanalytical chemistry, Drug Discovery, LCMS/MS	I have conceived the idea, planned all of the experimentation, contributed in data analysis and MS writing. I am also serving as a main supervisor to PhD student Ms. Khan.

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Dr. Mazhar Iqbal (DCS)
(Corresponding Author)

Institutional Review Committee

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[Signature]

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[Signature]

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[Signature]
16/6/20