Suplementation of Nigella sativa as Antioxidant in COVID-19 Patients

by

ORIGINAL ARTICLE

Suplementation of *Nigella sativa* as Antioxidant in COVID-19 Patients: *In silico* Study *via* the Nrf2-Keap1 Pathway

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ABSTRACT

The human corona virus disease of 2019 is a viral disease that can produce oxidative stress due to reduced antioxidant activity. Black cumin is a plant that can be taken as a supplement to boost antioxidant levels in the body, although the process is still unknown. As a result, the *in silico* method will be used to screen the potential of *Nigella sativa* peptide as an antioxidant in this study. Protein tracking was done using the UniProt database (https://www.uniprot.org/), with KEAP1 as the target protein (PDP: 5CGJ). Molecular docking was performed using Patchdock Server and antioxidant activity was determined using https://services.healthtech.dtu.dk/service.php?AnOxPePred-1.0. The researchers concluded that peptides found in *N. sativa*'s NAD(P)H-quinone oxidoreductase subunit 5, chloroplastic protein, had antioxidant potential through suppressing KEAP1 activity with the lowest ACE in Tyr-Tyr-Glu and Cys-Tyr-Tyr.

Keywords: COVID-19, KEAP1, *Nigella sativa*, Nrf2, Peptide.

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INTRODUCTION

Human coronavirus disease 2019 (COVID-19) is an infectious disease caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), a single-chain RNA virus that has a capsule, nucleocapsid, spike glycoprotein, and other non-structural proteins.[1,2] There were 4,260,677 confirmed COVID-19 patients in Indonesia between March 2, 2020 and December 20, 2021, with 144,013 deaths.[2]

COVID-19 infection is caused by viruses that enter cells and increase oxygen consumption, causing hypoxia in the cells, which causes oxidative stress and increased activity of antioxidant enzymes like peroxidase, catalase, and superoxide dismutase. Furthermore, oxidative stress can activate a number of transcription factors, including nuclear factor kappa-B (NF κB), p53, HIF-hypoxia-inducible factor 1α, peroxisome proliferator-activated receptor γ (PPAR-γ), β-catenin/Wnt, and Nrf2.[3,4]

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>tr|A0A161GVV5|A0A161GVV5_NIGSA NAD(P)H-quinone oxidoreductase subunit 5,
chloroplastic (Fragment) OS=Nigella sativa OX=555479 GN=ndhF PE=3 SV=1
GDFGLFLGILGLYWITGSFEFRDLFEITNNLVDNNETSSLFLILCAFLLFVGTVAKSAQF
PLHVWLPDAMEGPTPISALIHAATMVAAGIFLVARLFPLFTAIPSIMNIISLVGIITILL
GATLALSQRDIKRSLAYSTMSQLGYIMLALGMGSYRAALFHLITHAYSKALLFLGSGSII
HSMETIVGYSPDKSQNMALMGGLTKYVPITKTSFLLGTLSLCGIPPLACFWSKDEILNDS
WLYSPIFAIIACFTAGLTAFYMFRMYLLTFEGHLNVNFQNYSGKKNNAFYSISIWGKRGP
ELLKNICIFSTMNNKEKASFLSKKACPIDGNGVRDMRRPFIINNFANKKISTYPYESDNT
MLLPLLLLVLFILFVGFIGIPFGYEESDILSRWLTPSINFLHSNSNSSFDWYEFLINAIF
SVSIASLGIFIASTLYGPAYSYFHNFNLINLFVKRGPRRIICDPIINGIYNWSYNRGYID
VFYAKTLTRGIRGLAELTYFFDKQVIDGITNGIGLSNFFVAEIIKYIGGGRISSYIFFYL
FY
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Figure 1: NAD(P)H-quinone oxidoreductase subunit 5, chloroplastic protein sequence of NS

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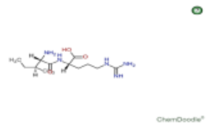
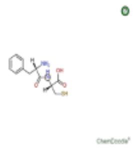
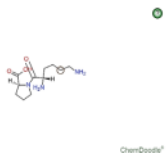
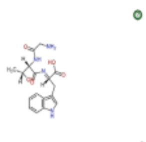
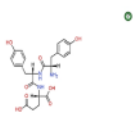
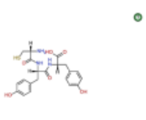
Table 1: Peptide antioxidant activity of NS

Peptides	*Structure*	*FRS Score*	*Chel Score*
Ile-Arg (IR)		0.38	0.25
Phe-Cys (KP)		0.41	0.28
Lys-Pro (FC)		0.43	0.27
Gly-Thr-Trp (GTW)		0.47	0.24
Tyr-Tyr-Glu (YYE)		0.58	0.21
Cys-Tyr-Tyr (CYY)		0.59	0.22

The erythroid 2-related nuclear factor protein (Nrf2) is a key protein in the Nrf2/KEAP1 pathway, which controls the antioxidant response. Nrf2 binds to the Kelch ECH Associating Protein 1 (KEAP1) protein when it is inactive. Free Nrf2, on the other hand, will translocate into the nucleus of the cell and trigger the expression of antioxidant genes like superoxide dismutase, catalase, and peroxidase.[3,4]

Many studies are currently being conducted on plants with antioxidant properties, such as *gemor, kelakai, bawang dayak, pasak bumi*, and others.[5-11] Many earlier researches have noted that nigella sativa (NS) is a plant that acts as an antioxidant. The administration of 500 mg/day of nigella sativa to rats exposed to cigarette acid was found to reduce serum oxidative damage (Table 1).[12] According to a study by Safithri *et al.*[13] NS at a dose of 4.8 g/kgBW/day for 8 weeks reduced oxidative damage in rats with hepatic fibrosis. Another study by Saleh *et al.*[14] stated that NS oil contains secondary metabolites such as pinene, thymoquinone, palmitic acid, oleic acid, linoleic acid, and thymol, which have a 16% antioxidant activity, but methanol extract only has a 12% antioxidant activity. Primary metabolites, such as peptide compounds present in NS, are considered to have antioxidant activity in addition to secondary metabolites, however this has not been well studied.

The antioxidant activity of NS has been studied extensively *in vitro* and *in vivo*. However, the mechanism of peptide compounds from NS acting as antioxidants in COVID-19 patients via the KEAP1-Nrf2 pathway has not been well investigated. Therefore, we conducted this research (Table 2).

MATERIAL AND METHOD

Protein Selection

Peptides in NS are derived from the breakdown of NAD protein (P) H-Quinone Oxidoreductase Subunit 5, Chloroplastic (UniprotKB-A0A161GVV5 code) in the Uniprot database (https://www.uniprot.org/). KEAP1 (GDP: 5CGJ) was chosen as the target protein from a data base protein (https://www.rcsb.org/).

Table 2: Results of molecular docking between peptides and KEAP1

Peptides	*Atomic contact energy (kJ/mol)*	*Hydrogen bonds*		*Hydrophobic bonds*	
		Residue	*Distance*	*Residue*	*Distance*
Ile-Arg (IR)	-75,69	Ser431 Gly433	2,41 3,12	Arg415 Ala556	4,35 4,50
Phe-Cys (KP)	-20,71	Val465 Val604	3,27 3,02	Ala466	4,61
Lys-Pro (FC)	-239,17	Val418 Val465 Val467 Val514	2,94 2,90 2,85 3,65	Cys513 Ala366	5,06 4,09
Gly-Thr-Trp (GTW)	-251,59	ASN414	2,72	ILE461 ARG415	5,32 4,32
Tyr-Tyr-Glu (YYE)	-262,85	Arg483 Arg415 Ser602	2,76 2,56 3,55	Tyr525 Tyr572	5,00 5,09
Cys-Tyr-Tyr (CYY)	-361,23	ASN382 Ser555	2,59 2,68	Tyr334	3,91

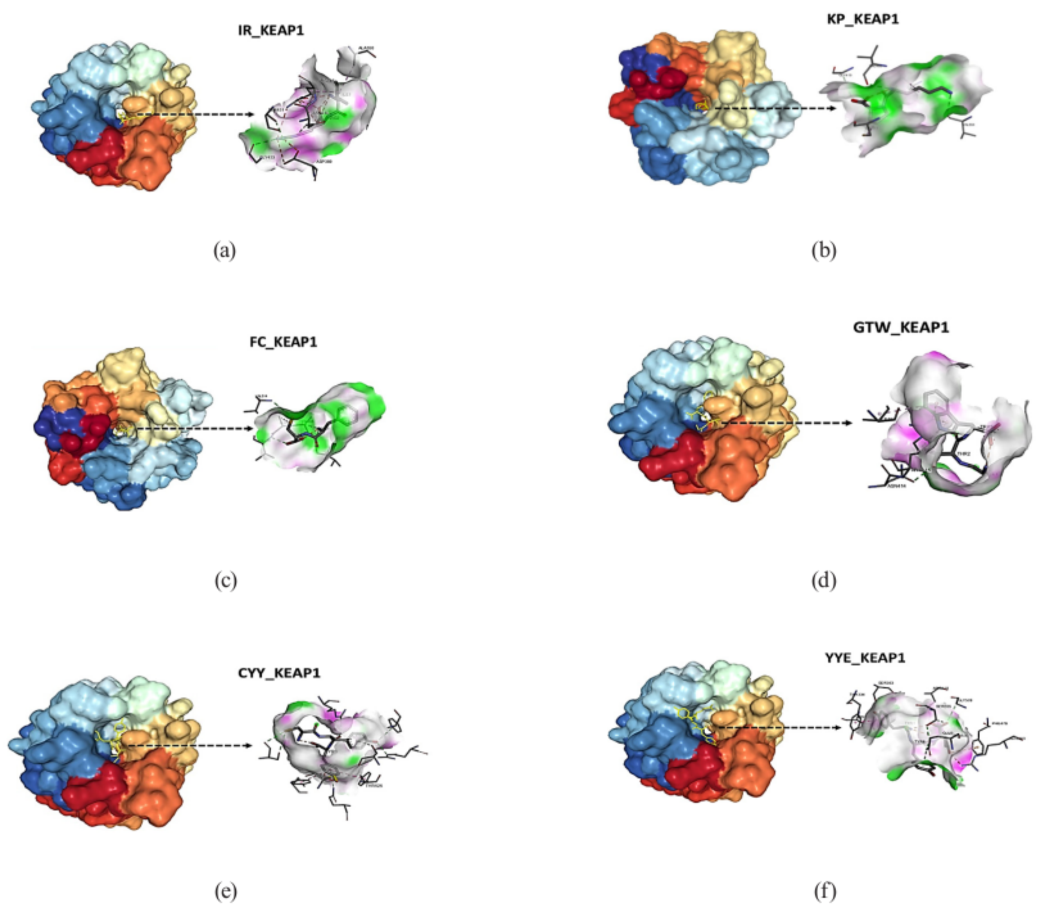


Figure 2: Interaction of KEAP1 protein with peptide (a) Ile-Arg (b) Phe-Cys (c) Lys-Pro (d) Gly-Thr-Trp (e) Tyr-Tyr-Glu and (f) Cys-Tyr- Tyr

Peptides Screening as Antioxidant

The website http://www.uwm.edu.pl/biochemia/index.php/en/biopep.[15] was used to screen bioactive peptides as antioxidants. Meanwhile, the Free Radical Scavenging Score and the Chelator Score were used to determine the level of antioxidant activity. The antioxidant activity score was obtained by visiting https://services.healthtech.dtu.dk/service.php?AnOxPePred-1.0.[16]

Molecular Docking

The Patchdock server is used for molecular docking. The Chimera 1.14 program is used to visualize the docking findings. Atomic Contact Energy, hydrogen bonds, and hydrophobic interactions between ligands and amino acid residues of receptor docking proteins will be presented.[17]

Toxicity and LD_{50}

Hepatotoxicity, carcinogenicity, immunotoxicity, and mutagenicity were all examined on the peptides that were obtained. Furthermore, the LD_{50} value is calculated. The website https://tox-new.charite.de/protox_II/index.php?site=home is used for testing.

RESULT

The enzyme NAD(P)H-quinone oxidoreductase subunit 5, chloroplastic, is present in the NS's respiratory chain. In this reaction, NADH is the reducing force that converts plastaquinone to plastaquinol. A chloroplastic protein from NS, NAD(P)H-quinone oxidoreductase subunit 5, has 542 amino acids. Figure 1 shows the outcomes of the protein sequence analysis.

The protein sequences were then screened for peptides that have antioxidant activity. The results are presented in Table 1.

The peptides in table 1 were then molecularly docked with KEAP-1 protein. The molecular docking results are presented in Table 2.

Visualization of molecular docking in Table 2 is presented in Figure 2.

Based on the results of the NS peptide toxicity test, the results are shown in Table 3.

DISCUSSION

Tyr-Tyr-Glu and Cys-Tyr-Tyr are NS peptides with a score greater than 0.5. (Table 1). This means that these peptides are capable of scavenging more than 50% of free radicals. The peptides Tyr-Tyr-Glu and Cys-Tyr-Tyr, on the other hand, have the lowest Atomic Contac Energy (ACE), indicating that the binding between the peptide and KEAP1 is strengthening.[17] As a result, the peptide appears to act by preventing the formation of the Nrf2-KEAP1 complex.[3,4]

Under basal conditions, Nrf2 is located in the cytoplasm and is inactive, which then binds to the repressor molecule Kelchlike ECH Association Protein 1 (KEAP1) to form the Nrf2-KEAP1 complex. KEAP1 is a protein with a molecular weight of 69-kDa protein which has a physiological function with Kelch protein as actin binder and acts as a negative regulator of Nrf.[3,4]

KEAP1 consists of several cysteine residues that act as sensors of intracellular redox status.[18] The ubiquitin proteosome pathway rapidly degrades Nrf2. Signals from ROS and electrophilic peptide compounds cause NRf2 to dissociate from KEAP1. NRf2 will then translocate to the nucleus. NRf2 binds to regulatory sequences known as antioxidant response elements or electrophile response elements (ARE/ApRE) in the promoter region of genes encoding antioxidants such as superoxide dismutase, catalase, peroxidase, and others in the nucleus. The inhibition of the Nrf2/KEAP1 pathway by NS peptides may have an effect on physiological function. This suggests that the NS peptide molecule acts as an antioxidant by interacting with KEAP1.[3,4]

The interaction formed between the NS peptides and the protein KEAP1 contributed to the ACE-indicated binding strength Table 2. onor/acceptor in protein ligands have a role. A low ACE level will improve the interaction between the peptide and the protein. The peptide-protein complex is stabilized by strong peptide and protein interactions. Low hydrophobicity improves compound permeability to the cell membrane and is inversely proportional to the amount of hydrophobic linkages.

Table 3 shows that, among the six peptides, Gly-Thr-Trp is a toxic peptide when compared to the other peptides. Meanwhile, Tyr-Tyr-Glu is a peptide with the potential to be a mutagen, or a chemical that can trigger gene alterations. Thus, in general, Cys-Tyr-Tyr is a peptide with antioxidant activity that is not hepatotoxic, carcinogenic, immunotoxic, mutagenic, or cytotoxic.

CONCLUSION

The peptides found in the NAD(P)H-quinone oxidoreductase subunit 5, chloroplastic protein of Nigella Sativa, exhibit antioxidant potential by suppressing Keap1 activity with the lowest ACE in Tyr-Tyr-Glu and Cys-Tyr-Tyr.

Table 3: Peptide toxicity test of NS

Peptides	*LD50 (mg/kg)*	*Hepatotoxicity probability*	*Carcinogenicity probability*	*Immunotoxicity probability*	*Mutagenicity probability*	*Cytotoxicity probability*
Ile-Arg	1000	0,9 inactive	0,67 inactive	0,99 inactive	0,71 inactive	0,69 inactive
Phe-Cys	5000	0,93 inactive	0,59 inactive	0,99 inactive	0,83 inactive	0,86 inactive
Lys-Pro	6800	0,88 inactive	0,83 inactive	0,99 inactive	0,58 inactive	0,69 inactive
Gly-Thr-Trp	800	0,8 inactive	0,75 inactive	0,99 inactive	0,69 inactive	0,64 inactive
Tyr-Tyr-Glu	5000	0,93 inactive	0,74 inactive	0,99 inactive	0,65 active	0,66 inactive
Cys-Tyr-Tyr	5000	0,81 inactive	0,75 inactive	0,98 inactive	0,82 inactive	0,76 inactive

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