



# Anthocyanin, tartaric acid, ascorbic acid of roselle flower (*Hibiscus sabdariffa* L.) for immunomodulatory adjuvant therapy in oral manifestation coronavirus disease-19: An immunoinformatic approach

[Antocianina, ácido tartárico, ácido ascórbico de flor de Jamaica (*Hibiscus sabdariffa* L.) para la terapia adyuvante inmunomoduladora en la manifestación oral de la enfermedad por coronavirus-19: Un enfoque inmunoinformático]

Nastiti Faradilla Ramadhani<sup>1</sup>, Alexander Patera Nugraha<sup>1,2\*</sup>, Desintya Rahmadhani<sup>3</sup>, Martining Shoffa Puspitaningrum<sup>3</sup>, Yuniar Rizqianti<sup>3</sup>, Viol Dhea Kharisma<sup>4</sup>, Tengku Natasha Eleena binti Tengku Ahmad Noor<sup>5</sup>, Rini Devijanti Ridwan<sup>6</sup>, Diah Savitri Ernawati<sup>7</sup>, Albertus Putera Nugraha<sup>8</sup>

<sup>1</sup>Graduate Student of Dental Health Science, Faculty of Dental Medicine, Universitas Airlangga, Surabaya, Indonesia.

<sup>2</sup>Department of Orthodontic, Faculty of Dental Medicine, Universitas Airlangga, Surabaya, Indonesia.

<sup>3</sup>Faculty of Dental Medicine, Universitas Airlangga, Surabaya, Indonesia.

<sup>4</sup>Graduate Student of Biology Science, Department of Biology, Faculty of Mathematics and Natural Science, Universitas Brawijaya, Malang, Indonesia

<sup>5</sup>Military Dental Officer of Royal Medical and Dental Corps, Malaysian Armed Forces, Indonesia.

<sup>6</sup>Department of Oral Biology, Faculty of Dental Medicine, Universitas Airlangga, Surabaya, Indonesia.

<sup>7</sup>Department of Oral Medicine, Faculty of Dental Medicine, Universitas Airlangga, Surabaya, Indonesia.

<sup>8</sup>Faculty of Medicine, Universitas Airlangga, Surabaya, Indonesia.

\*E-mail: [alexander.patera.nugraha@fkg.unair.ac.id](mailto:alexander.patera.nugraha@fkg.unair.ac.id)

## Abstract

**Context:** Oral manifestations that arose from COVID-19 infection often causes morbidity and systemic drug administration is less effective. Roselle flower (*Hibiscus sabdariffa*) is one of the plants that is often used in infusion as it gives health benefits. Hence, *H. sabdariffa* may benefit from adjuvant therapy to treat oral manifestation due to COVID-19.

**Aims:** To investigate the potential of *H. sabdariffa* anthocyanins, tartaric acid, and ascorbic acid chemical compounds as antiviral, anti-inflammatory, antioxidant, and increasing tissue regeneration in oral manifestation due to COVID-19 infection *in silico*.

**Methods:** Chemical compounds consisted of anthocyanins, (+)-tartaric acid, and ascorbic acid beside target proteins consisted of ACE2-spike, Foxp3, IL-10, IL6, IL1 $\beta$ , VEGF, FGF-2, HSP70, TNFR and MDA-ovalbumin were obtained from the database, ligand samples were selected through absorption, distribution, metabolism, excretion and toxicology analysis, then molecular docking simulations, identification of protein-ligand interactions, and 3D visualization were performed.

**Results:** Anthocyanins, tartaric acid, and ascorbic acid are the active compounds in *H. sabdariffa*, which act as antioxidants. The activity of anthocyanin compounds is higher than other compounds through value binding affinity, which is more negative and binds to specific domains of target proteins by forming weak binding interactions that play a role in biological responses. Anthocyanins have the most negative binding energy compared to tartaric-acid and ascorbic acid.

**Conclusions:** Anthocyanins act as antioxidants; this mechanism increases heat shock protein-70 (HSP70), which may play an important role in increasing wound regeneration of oral manifestation in severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) as documented *in silico*.

**Keywords:** COVID-19; dentistry; *Hibiscus sabdariffa*; infectious disease; medicine.

## Resumen

**Contexto:** Las manifestaciones orales derivadas de la infección por COVID-19 suelen causar morbilidad y la administración sistémica de fármacos es menos efectiva. La flor de Jamaica (*Hibiscus sabdariffa*) es una de las plantas que se suele utilizar en infusión ya que aporta beneficios para la salud. Por lo tanto, *H. sabdariffa* puede beneficiarse de la terapia adyuvante para tratar las manifestaciones orales debido a COVID-19.

**Objetivos:** Investigar el potencial de los compuestos químicos de *H. sabdariffa*, como antocianinas, ácido tartárico y ácido ascórbico como antivirales, antiinflamatorios, antioxidantes y el aumento de la regeneración de tejidos en la manifestación oral debido a la infección por COVID-19 a través de un enfoque inmunoinformático, un estudio *in silico*.

**Métodos:** Antocianinas, ácido tartárico y ácido ascórbico, además de proteínas diana como ACE2-spike, Foxp3, IL-10, IL6, IL1 $\beta$ , VEGF, FGF-2, HSP70, TNFR y MDA-ovoalbúmina, se obtuvieron de la base de datos, las muestras de ligando se seleccionaron mediante análisis de absorción, distribución, metabolismo, excreción y toxicología, luego se realizaron simulaciones de acoplamiento molecular, identificación de interacciones proteína-ligando y visualización 3D.

**Resultados:** Las antocianinas, el ácido tartárico y el ácido ascórbico son los compuestos activos de *H. sabdariffa* que actúan como antioxidantes. La actividad de los compuestos de antocianina es mayor que la de otros compuestos a través de una afinidad de unión de valor que es más negativa y se une a dominios específicos de proteínas diana formando interacciones de unión débiles que desempeñan un papel en las respuestas biológicas. Las antocianinas tienen la energía de unión más negativa en comparación con el ácido tartárico y el ácido ascórbico.

**Conclusiones:** Las antocianinas actúan como antioxidantes; este mecanismo aumenta la proteína de choque térmico-70 (HSP70), que puede desempeñar un papel importante en el aumento de la regeneración de heridas de la manifestación oral en el síndrome respiratorio agudo severo coronavirus 2 (SARS-CoV-2) como se documenta *in silico*.

**Palabras Clave:** COVID-19; enfermedad infecciosa; *Hibiscus sabdariffa*; medicamento; odontología.

## ARTICLE INFO

Received: November 23, 2021.

Received in revised form: January 22, 2022.

Accepted: January 25, 2022.

Available Online: February 6, 2022.

## INTRODUCTION

Coronavirus disease 2019 (COVID-19) is a disease that has occurred since late 2019 in Wuhan, Hubei Province, China, caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) (WHO, 2021). Indonesia ranks 16<sup>th</sup> globally and 4<sup>th</sup> in Asia in the number of COVID-19 cases (WHO, 2021). The most common symptoms found in COVID-19 patients are dry cough, fever, and shortness of breath; however, it may vary in patients. Dentists play an important role in examining and managing oral manifestations of COVID-19 found on intraoral examination (Iranmanesh et al., 2021).

Many drugs, such as remdesivir, chloroquine, and hydroxychloroquine, are currently used to treat COVID-19 patients (Gani et al., 2021). A poorly understood immune malfunction has resulted in an uncontrolled inflammatory response, which has been the hallmark of severe COVID-19 illness. Previous research using cytometric and transcriptome profiling indicated a unique regulatory T cells (Tregs) profile in severe COVID-19 patients, with a rise in Treg proportions and intracellular levels of the lineage-defining transcription factor forkhead box P3 (FoxP3), which was linked to poor outcomes. Tregs may play sinister roles in COVID-19, inhibiting antiviral T cell responses during the disease's severe phase and acting as a direct pro-inflammatory factor (Galvan-Pena et al., 2020). Interleukin (IL) IL-6 levels are considerably higher in COVID-19 patients and are related with poor clinical outcomes. Inhibiting IL-6 may be a unique therapeutic target for controlling dysregulated host responses in COVID-19 patients (Coomes and Haghbayan, 2020). The development of COVID-19 has been linked to dysregulation of IL-1 $\beta$  and IL-6 responses but not cytokine transcripts themselves, especially in the nasopharynx and blood. However, it is not linked with COVID-19 disease severity, length of stay, or death. The transcriptional response modules for IL-1 $\beta$  and IL-6 give a dynamic readout of functional cytokine activity *in vivo*, which aids in quantifying the biological effects of immunomodulatory therapy in COVID-19 (Bell et al., 2021).

Besides, there was an increase in tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) levels in COVID-19 patients. Thus, this condition leads to neutrophil chemotaxis in the oral mucosa and develops into aphthous lesions. The inflammatory response in COVID-19 patients is im-

proved with immunoregulation when the inflammation takes place and is considered as a potential therapy for the oral manifestations of COVID-19 (Diaz Rodriguez et al., 2020; Iranmanesh et al., 2021).

Levels of vascular endothelial growth factor (VEGF) and fibroblast growth factor-2 (FGF-2) in COVID-19 patients dramatically rise with disease severity (Smadja et al., 2021). VEGF, a key factor in angiogenesis, can stimulate endothelial cell migration and proliferation, resulting in tissue regeneration (Diomedea et al., 2020). On the other hand, VEGF has been linked to inflammation and the pathophysiology of vasculitic diseases. Increased VEGF expression in oral aphthous lesions in connection with a cluster of differentiation (CD) CD34 positivity may indicate a role for VEGF in the development of these vasculitic lesions. VEGF may be involved in the progression of oral aphthous lesions (Yalçin et al., 2006). FGF-2 is a crucial angiogenesis mediator that is required for proper reproduction and wound repair. FGF-2 promotes angiogenesis by attaching to heparin sulfate proteoglycan as well as a tyrosine kinase receptor. FGF-2 promotes cell attachment via interactions with two FGF receptor-1 isoforms as well as extracellular matrix or cell-associated heparin sulfate proteoglycans (Plum et al., 2004). Previous research found that FGF-2 paste is effective in relieving ulcer pain and encouraging healing without causing severe adverse effects in the treatment of mild recurring aphthous stomatitis (Jiang et al., 2013).

Cytokine storm has an important role in the pathophysiology of COVID-19. The link between inflammation and oxidative stress has been extensively established. Malondialdehyde (MDA), an oxidative stress measure, is considerably higher in COVID-19 patients. This might have a crucial role in disease development mechanisms. Patients with COVID-19 may benefit from methods for lowering or avoiding peroxidation, such as the use of an antioxidant substance (Mehri et al., 2021). Oxidative stress is causing an increase in heat shock proteins (HSPs) expression. HSPs are intracellular proteins that function as molecular chaperones in protein folding and intracellular protein trafficking. Oxidative stress is causing an increase in HSP expression. A relative deficit of heat shock response (HSR) is one of the unique pre-existing comorbidities linked with severe COVID-19 infection. A lack of adequate HSR has been indicated to be the likely underlying etiology for the poor prog-

nosis, and it is also suspected in groups at high risk of COVID-19 mortality (Heck et al., 2020; Danladi and Sabir, 2021).

Necroptosis, a caspase-independent programmed apoptosis, is critical in the course of a number of inflammatory diseases. Necroptosis is also implicated in the extracellular exposure of numerous damage-associated molecular patterns (DAMPs), including HSPs, which leads to inflammation. Endocytosed or intracellular HSP70 is presented by major histocompatibility complex (MHC)-II molecules and, in the absence of proper co-stimulation, leads to the expansion of tolerogenic or Treg responses, which have inflammation suppressive activity through the production of anti-inflammatory cytokines, the suppression or killing of effector T cells, or the conversion of antigen presenting cells (APC) to a tolerogenic state (Khandia et al., 2017). HSP-caused during COVID-19 infection, Tregs play a key role in the cytokine storm. A previous study discovered that blood concentrations of both HSP 27 and HSP 70 were considerably higher in seriously damaged individuals (Haider et al., 2019).

Natural ingredients are proven to have the ability to control infectious diseases (Babich et al., 2020). In several countries, including Indonesia, the roselle flower (*Hibiscus sabdariffa* L., family *Malvaceae*) is one of the medicinal plants that can be easily found and consumed. The chemical components present in *H. sabdariffa* have potential health benefits, including antiseptic, diuretic, antihypertensive, anticholesterol, antibacterial, and antioxidant. The content of ascorbic acid and flavonoids make this plant have natural antioxidant activity, which can act as an immunoregulatory agent (Shruthi et al., 2016; Syahrana et al., 2017). Therefore, this study is aimed to identify the potential of *H. sabdariffa* chemical compounds such as anthocyanins, (+)-tartaric acid, and ascorbic acid as antiviral, anti-inflammatory, antioxidant, and increase tissue regeneration in oral manifestation due to COVID-19 infection through an immunoinformatics approach, an *in silico* study.

## MATERIAL AND METHODS

### Sample retrieval and absorption, distribution, metabolism, excretion and toxicology analysis

The chemical compounds containing *H. sabdariffa* used in this study consisted of anthocyanins, (+)-tartaric acid, and ascorbic acid (vitamin C) obtained from PubChem (<https://pubchem.ncbi.nlm.nih.gov/>) (Carvajal-Zarrabal et al., 2012). The samples were identified by ID, formula, weight, and smile canonical; then, the ligand structure was minimized in PyRx software to increase flexibility (Kharisma et al., 2020).

Preparations were carried out on The Research Collaboratory for Structural Bioinformatics Protein Data Bank (RCSB PDB) (<https://www.rcsb.org/>) protein consisting of angiotensin-converting enzyme-2 (ACE-2)-spike, interleukin (IL)-10, forkhead box P3 (Foxp3), IL6, IL1-B, tumor necrosis factor receptor (TNFR), vascular endothelial growth factor (VEGF), fibroblast growth factor-2 (FGF-2), heat shock protein-70 (HSP-70), and malondialdehyde (MDA)-ovalbumin, information related to the targeted protein obtained from the database consists of ID, visualization method, resolution, atom count, weight, chain, and sequence length, then sterilization of protein samples is carried out through PyMol software for molecular docking optimization (Kharisma et al., 2021). Druglikeness was carried out on the three chemical compounds containing *H. sabdariffa* by referring to Lipinski's rules (<http://www.scfbio-uitd.res.in/software/drugdesign/lipinski.jsp>) and ADME analysis (<http://www.swissadme.ch/>).

### Docking simulation

The activity of ligand binding to the target protein domain was identified by molecular docking simulation. It aims to determine the type of activity inhibiting and enhancing the activity of the target. The binding energy produced by the ligand when it binds to the target protein site can trigger a specific biological response, the more negative the binding score, and the higher the effect on target protein activity (Luqman et al., 2020). The ligands in this study were three compounds containing *H. sabdariffa*, and the target protein consisted of ACE2-spike, IL-10, Foxp3, IL6, IL1B, VEGF, FGF-2, HSP70, TNFR, and MDA-ovalbumin.

### Ligand-protein interaction

Identification of the type of activity of the target protein triggered by the two ligands is known through the analysis of the position and chemical bonds in the molecular complex resulting from molecular docking in the Discovery Studio software. Hydrophobic, hydrogen, pi, and Van der Waals bonds formed in molecular complexes can be identified through the server. The type of weak bond interactions can play a role in the context of the biological activity of a protein (Putra et al., 2020; Widyananda et al., 2021).

### Molecular visualization

This research uses PyMol software for 3D visualization of molecular docking results. The 3D structure of the ligand-protein molecular complex is shown in the form of cartoons, surfaces, sticks, and spheres (Susanto et al., 2018).

## RESULTS

Ligand samples consisting of anthocyanins, (+)-tartaric acid, and ascorbic acid (vitamin C) were obtained with information on ID, formula, weight, and canonical smile. All of the bioactive compounds containing *H. sabdariffa* were classified as drug candidates based on Lipinski and ADME predictions (Tables 1 and 2). Protein samples consisting of ACE2-spike, IL-10, Foxp3, IL6, IL1B, VEGF, FGF-2, HSP70, TNFR, and MDA-ovalbumin were obtained from the RCSB database. PDB was obtained with information on ID, visualization method, resolution, atom count, weight, chain, and sequence length (Table 3). Based on the results of molecular docking simulations, anthocyanins containing *H. sabdariffa* have the most negative binding energy compared to other compounds. This

indicates that anthocyanins were predicted to affect the activity of target proteins, such as inhibition and enhancement (Table 4). All docked protein complexes were shown with cartoons structure, transparent surfaces, and colored selection based on their constituent structures, then for ligands displayed with sticks structure (Fig. 1). Identification of molecular interactions and binding positions on the docked protein-ligand complex (Fig. 2) showed that the binding of anthocyanins to all target proteins resulted in non-covalent bond interactions consisting of Van der Waals, pi, and hydrogen (Table 5). These interactions contribute to the formation of the protein-ligand complex and initiate the formation of an activity response on the target protein consisting of enhancement and inhibition.

**Table 1.** Results of ligand sample preparation and drug-likeness.

Compound	CID	Formula	Molecular weight (Dalton)				
			(>500)	LOGP (>5)	HBD (>5)	HBA (>10)	MR (40-130)
Anthocyanin	145858	C <sub>15</sub> H <sub>11</sub> O <sup>+</sup>	207.000	4.190	0	1	65.395
(+)-Tartaric acid	444305	C <sub>4</sub> H <sub>6</sub> O <sub>6</sub>	150.000	-2.122	4	6	27.285
Ascorbic acid (vit C)	54670067	C <sub>6</sub> H <sub>8</sub> O <sub>6</sub>	176.000	-1.407	4	6	35.256

**Table 2.** ADME analysis results.

Compound	Water solubility (Log S) subhead	Pharmacokinetics subhead	Topological surface (Å)
Anthocyanin	-3.47/Highly soluble	GI absorption: High Synthetic accessibility: 2.78	13.14
(+)-Tartaric acid	-0.02/Very soluble	GI absorption: Low Synthetic accessibility: 2.57	115.06
Ascorbic acid	-0.10/Soluble	GI absorption: High Synthetic accessibility: 3.47	107.22

**Table 3.** Results of preparation of target protein samples from RCSB PDB.

Name	PDB ID	Visualization method	Resolution (Å)	Atom count	Weight (kDa)	Chain	Sequence length (mer)
ACE-Spike	7KJ2	Electron microscopy	3.60	28560	495.88	A,B,C,D	1234 & 615
IL-10	1INR	X-Ray	2.00	1114	18.67	A	160
Foxp3	4WK8	X-Ray	3.40	2243	33.05	C,D	82
IL6	1IL6	NMR	-	166	21.01	A	185
IL1B	4G6J	X-Ray	2.03	4769	64.49	A	158
TNFR	1EXT	X-Ray	1.85	2887	36.98	A,B	2887
VEGF	2VPF	X-Ray	1.93	6782	95.59	A,B	102
FGF-2	1BLA	NMR	-	1221	17.35	A	155
HSP70	1S3X	X-Ray	1.84	3375	42.75	A	382
MDA-ovalbumin	1OVA	X-Ray	1.95	12269	172.44	A	386



**Table 4.** Molecular docking simulation results.

Target	Grind position			Binding affinity (kcal/mol)
	Ligand	Centre	Dimension	
ACE-spike	Anthocyanin	X: 206.507	X: 102.969	-7.5
	(+)-Tartaric acid	Y: 179.855	Y: 106.384	-4.9
	Ascorbic acid	Z: 202.947	Z: 190.431	-5.4
IL-10	Anthocyanin	X: 12.458	X: 55.622	-7.6
	(+)-Tartaric acid	Y: 21.380	Y: 38.549	-4.2
	Ascorbic acid	Z: 4.399	Z: 66.373	-5.2
Foxp3	Anthocyanin	X: -14.567	X: 47.543	-7.1
	(+)-Tartaric acid	Y: -5.463	Y: 53.499	-4.7
	Ascorbic acid	Z: -5.627	Z: 49.495	-4.5
IL6	Anthocyanin	X: 13.025	X: 45.583	-7.5
	(+)-Tartaric acid	Y: 21.380	Y: 38.549	-4.2
	Ascorbic acid	Z: 4.399	Z: 66.373	-5.2
IL1B	Anthocyanin	X: 18.487	X: 41.087	-6.5
	(+)-Tartaric acid	Y: -12.108	Y: 41.866	-4.7
	Ascorbic acid	Z: -36.277	Z: 37.665	-5.5
TNFR	Anthocyanin	X: -3.833	X: 92.579	-7.1
	(+)-Tartaric acid	Y: 32.637	Y: 67.184	-5.0
	Ascorbic acid	Z: -4.686	Z: 68.334	-5.8
VEGF	Anthocyanin	X: -7.420	X: 52.634	-6.0
	(+)-Tartaric acid	Y: -1.430	Y: 48.545	-3.5
	Ascorbic acid	Z: -4.507	Z: 39.120	-4.8
IL1B	Anthocyanin	X: 18.487	X: 41.087	-6.5
	(+)-Tartaric acid	Y: -12.108	Y: 41.866	-4.7
	Ascorbic acid	Z: -36.277	Z: 37.665	-5.5
TNFR	Anthocyanin	X: -3.833	X: 92.579	-7.1
	(+)-Tartaric acid	Y: 32.637	Y: 67.184	-5.0
	Ascorbic acid	Z: -4.686	Z: 68.334	-5.8
VEGF	Anthocyanin	X: -7.420	X: 52.634	-6.0
	(+)-Tartaric acid	Y: -1.430	Y: 48.545	-3.5
	Ascorbic acid	Z: -4.507	Z: 39.120	-4.8

## DISCUSSION

COVID-19 is an infectious disease that triggers inflammatory responses and cell death apart that it also involves immunopathogenesis (Li et al., 2020). Manifestations of COVID-19 that arise due to disturbance of immunity, such as lymphopenia caused by decreasing expression of T cells and natural killer cells (NK-Cells), as well as the appearance of oral manifestations due to cytokine storm in COVID-19, namely a significant increase in the expression of IL-1 $\beta$ , IL-6, IL-10, monocyte chemoattractant protein-1 (MCP-

1/CCL2), granulocyte colony-stimulating factor (G-CSF), TNF- $\alpha$ , macrophage inflammatory protein-1 alpha (MIP-1 $\alpha$ /CCL3) (Luo et al., 2021; López-Collazo et al., 2020)

These conditions can trigger oral lesions such as erosion and ulceration as a typical manifestation of COVID-19. Increased expression of neutrophils decreased lymphocyte activity, and increased antibody-dependent activity can trigger the appearance of aphthous like ulcers, geographic tongue, and petechiae in COVID-19 patients (Amorim Dos Santos et al., 2020; Vieira et al., 2021). Immunomodulation may be a po-

tential therapy for COVID-19 as pegylated interferon (IFN) alpha-2a and 2b can stimulate the innate antiviral response of SARS-CoV-2 that infects the host (Velavan and Meyer, 2020).

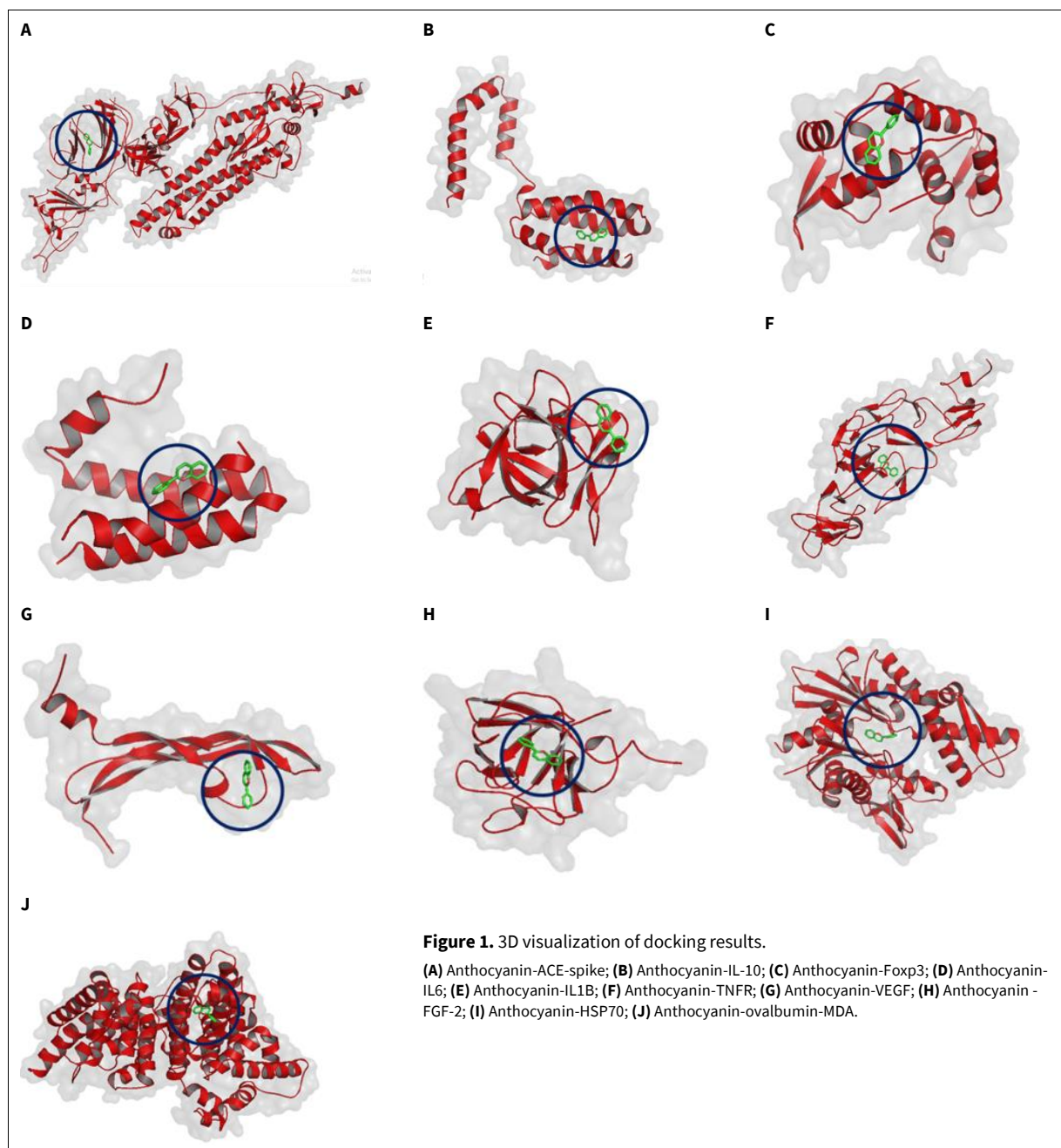
Various herbal plants have been discovered to have great potential as alternative medicine and are the basis for the discovery of natural compounds for the development of therapeutic agents in the field of pharmacology. It is known that the lungs are the organ that is the most severely affected by COVID-19 virus, similar to SARS-CoV-2 infection. Therefore, special attention is paid to herbal plants that can protect the lungs and support the immune system (Babich et al., 2020). *H. sabdariffa* is one of the plants whose all parts have functional benefits for the human body, including as an immunomodulator in conditions infected with SARS-CoV-2 (Marpaung, 2020). Ethanol and aqueous extracts from *H. sabdariffa* flower petals have immunomodulatory functions by increasing the production of IL-10 and decreasing the production of pro-inflammatory cytokines such as TNF- $\alpha$  and IL-6 (Izquierdo-Vega et al., 2020). Cytokine storms are very common in severe COVID-19 patients, which are characterized by the excessive and uncontrolled release of pro-inflammatory cytokines (Dewi et al., 2021). It is suggested *H. sabdariffa* may prevent the occurrence of cytokine storms in COVID-19 patients through the immunomodulation mechanism.

Research on the safety of *H. sabdariffa* conducted by Sari et al. (2016) stated that the lethal dose 50 (LD<sub>50</sub>) of *H. sabdariffa* ethanol extract was 850.90 mg/kg body weight against Sprague Dawley rats (classified as mildly toxic, LD50 >500 - 5000 mg) (Sari et al., 2016). The preclinical test results above are needed to proceed to the clinical trial phase to find the effect of *H. sabdariffa* flower capsules at a dose of 500 mg/day for 30 days on IL-10 expression in healthy volunteers. The results of research conducted by Syahrana et al. (2017) stated that administration of capsules of *H. sabdariffa* flower powder at a dose of 500 mg/day for 30 days had an effect on increasing the expression of IL-10, but within normal limits in healthy volunteers. The immunostimulatory effect of *H. sabdariffa* is due to the content of phenolic compounds and flavonoids (flavonols and anthocyanins) (Syahrana et al., 2017).

The molecular docking method used in this study was blind to screen the binding of a ligand to determine the activity response to the target protein (Kharisma et al., 2020). The results of molecular docking simulations show that the greater the binding affinity produced by a ligand when it binds to a specific domain of the target protein, the more negative it can increase protein activity, and this activity refers to the research objectives (Widyananda et al., 2021). Binding affinity is an energy produced by a complex of protein molecules and ligands when they bind through a docking simulation (Luqman et al., 2020).

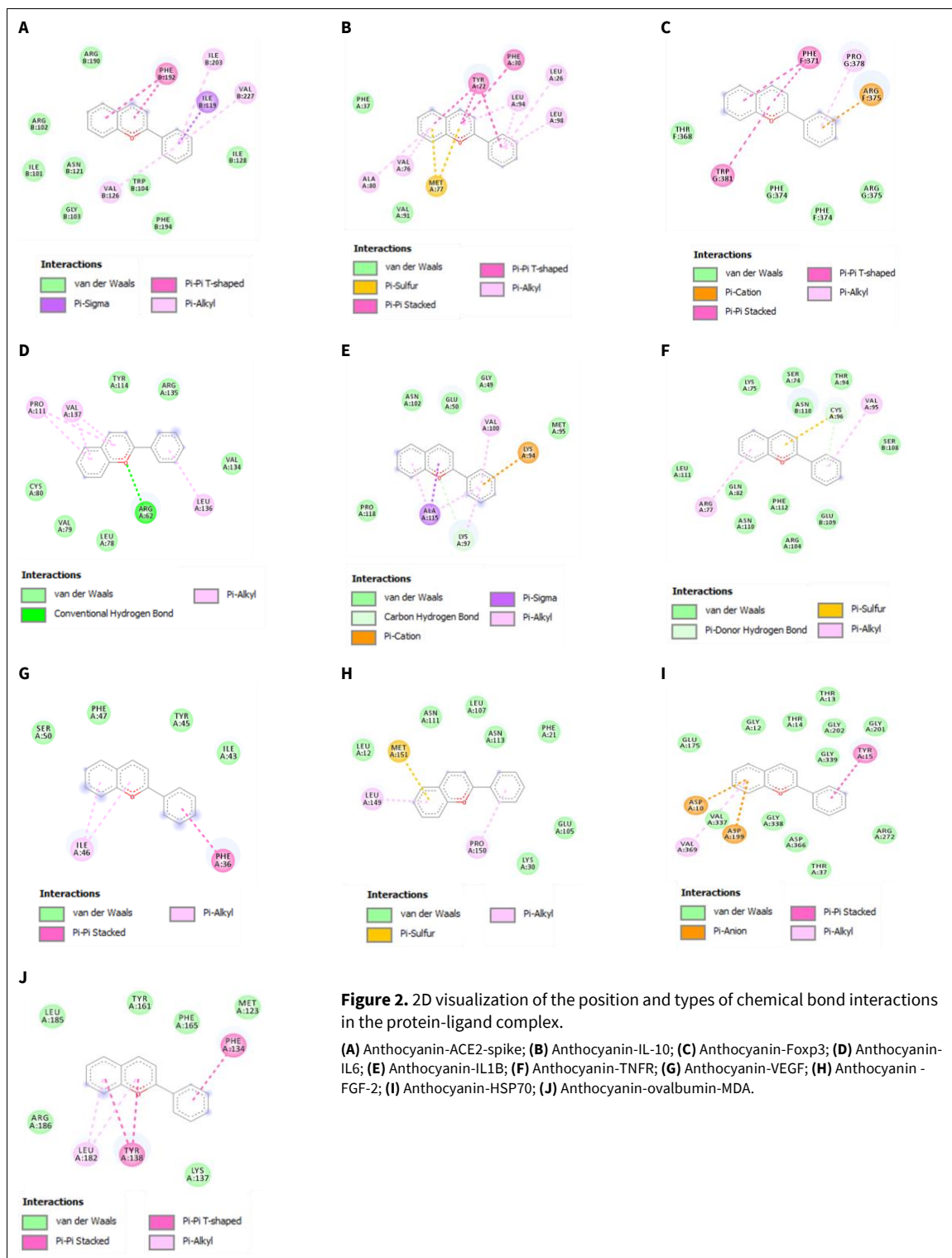
**Table 5.** Results of identification of molecular interactions.

Molecular complex	Molecular interaction
Anthocyanin-ACE2-Spike	<b>Van der Waals:</b> Arg120, Arg102, Ile128, Ile101, Asn121, Gly103, Trp104, Phe194 <b>Pi:</b> Ile203, Phe192, Ile119, Val227
Anthocyanin-IL-10	<b>Van der Waals:</b> Phe37, Val91 <b>Pi:</b> Ala80, Val76, Met77, Tyr72, Phe30,
Anthocyanin-Foxp3	<b>Van der Waals:</b> Thr368, Phe374, Arg375 <b>Pi:</b> Phe371, Trp381, Pro378, Arg375
Anthocyanin-IL6	<b>Pi:</b> Leu94, Leu26, Leu98
Anthocyanin-IL1B	<b>Pi:</b> Val100, Ala115 <b>Hydrogen:</b> Lys97
Anthocyanin-TNFR	<b>Pi:</b> Arg77, Cys96, Val95 <b>Hydrogen:</b> Cys96
Anthocyanin-VEGF	<b>Van der Waals:</b> Ser50, Phe47, Tyr45, Ile43 <b>Pi:</b> Ile46, Phe36
Anthocyanin-FGF-2	<b>Van der Waals:</b> Leu12, Asn111, Leu107, Asn113, Phe21, Glu105, Lys30 <b>Pi:</b> Met151, Leu149, Pro150
Anthocyanin-HSP70	<b>Pi:</b> Asp10, Val369, Asp199, Tyr15
Anthocyanin-ovalbumin-MDA	<b>Van der Waals:</b> Leu185, Tyr161, Phe165, Met123, Arg186, Lys137 <b>Pi:</b> Leu182, Tyr138, Phe134



Based on the results of molecular docking (*in silico*), *H. sabdariffa* flower extract contains anthocyanin, (+)-tartaric acid then compared with ascorbic acid against several markers such as the glycoprotein spike SARS-CoV-2 and hACE-2, IL-10, Foxp3, IL-6, IL1BR, TNFR, VEGF, FGF-2, HSP-70, ovalbumin. The results showed that *H. sabdariffa* flower extract could inhibit the binding between ACE-2 and spike SARS-CoV-2 and the binding of TNF-R with TNF- $\alpha$ . In addition, *H. sabdariffa* can increase IL-10, Foxp3, which are anti-inflammatory markers. In the results, from molecular docking (*in silico*). It was also found that *H. sabdariffa* flower extract can reduce the secretion of pro-inflammatory cytokines such as IL-6 and IL1, but increase VEGF and FGF-2, which are markers of neovascularization, anthocyanin also act as antioxidants

through an increase in HSP-70 and ovalbumin, also decrease MDA. Anthocyanins more inhibited the binding of ACE-2 to the SARS-CoV-2 spike than tartaric acid and ascorbic acid. Anthocyanins are more effective in enhancing IL-10, VEGF, FGF-2, HSP-70, Foxp3, ovalbumin, and decrease IL-1B, IL6, TNFR compared to tartaric acid and ascorbic acid. By inhibiting the binding of ACE 2 to the SARS-CoV-2 spike, anthocyanins have the potential as antivirals during the virus attachment process. In addition, anti-inflammatory potential of anthocyanins through the mechanism of increasing IL-10 and Foxp3 cytokines in macrophages and inhibiting TNF $\alpha$ -TNFR binding. This allows a decrease of pro-inflammatory cytokines such as IL-6. Anthocyanin is one of the compounds in



*H. sabdariffa* flower, which has the ability as an immunomodulator. Anthocyanins can regulate the immune system, which has decreased in SARS-CoV-2 infected patients (Al-Snafi, 2016). Besides, anthocyanins are able to act as anti-inflammatory and antioxidants, which may overcome the oral manifestations of COVID-19 due to the inflammatory response, which

also supports the immunomodulating activity (Al-Snafi, 2016; Gollen et al., 2018).

Anthocyanins allow to generate an antiviral response through inhibition of the SARS-CoV-2, an ACE-glycoprotein complex during the viral attachment process because it binds to the ACE domain precisely blocking the Receptor Binding Domain (RBD) on the SARS-CoV-2 spike glycoprotein. Then,



the anti-inflammatory potential is also possessed by anthocyanins through the mechanism of stimulating the increase in IL-10 and Foxp3 cytokines in macrophages. Anthocyanins also inhibit the binding of TNF $\alpha$  via TNFR. This allows a decrease in the release of pro-inflammatory cytokines and inhibition of IL-6 is also possible through M1/M2 polarization. Anthocyanins contained in *H. sabdariffa* are predicted to trigger an increase in tissue regeneration through stimulation of VEGF and FGF-2, then anthocyanin compounds also increase the activity of the HSP70 chaperon protein and reduce MDA through ovalbumin as an antioxidant biomarker. The weak bond interactions identified in the docking complex play a role in initiating the formation of specific biological responses. These interactions are played by hydrogen bonds, hydrophobicity, Van der Waals, and pi. Weak bonds are also possible to trigger the stability of the ligand-protein binding complex so that specific responses can run (Susanto et al., 2018; Widyananda et al., 2021).

Anti-inflammatory and antioxidant properties of *H. sabdariffa* flower play an important role in immunomodulatory activity (Fakeye, 2008; Mishra et al., 2012). The intensity of the inflammatory response is controlled by the recruitment of inflammatory cells into the inflammatory lesion (Zhang and An, 2007). The role of chemokines and adhesion molecules in modulating the immune response, leading to a substantial disturbance of mediators' expression, leads to the enhancement or inhibition of the immune response (Sokol and Luster, 2015). The *H. sabdariffa* flower mediates its inhibitory effect on the inflammatory immune response through modulation of the expression of chemokines and adhesion molecules, as well as inhibition of humoral antibody formation and phagocytic ability (Umeoguaju et al., 2021).

In COVID-19 therapy, it is often associated with providing nutrition in the form of ascorbic acid. Ascorbic acid accumulates in phagocytic cells, such as neutrophils, and can promote chemotaxis, phagocytosis, generate reactive oxygen species, and ultimately kill microbes (Arrigoni and De Tullio, 2002). Ascorbic acid can also remove strong free radicals in plasma, protecting cells against oxidative damage caused by reactive oxygen species (ROS) (Reang et al., 2021). Furthermore, it can reduce the inflammatory reaction by inhibiting TNF- $\alpha$  and nuclear factor  $\kappa$ B, which are the main pro-inflammatory transcription factors (Atiqi et al., 2020).

Based on this present study, anthocyanins in *H. sabdariffa* flowers are predicted to trigger an increase in tissue regeneration through stimulation of VEGF and FGF-2 and increase the activity of the HSP70 chaperon protein and reduce MDA through ovalbu-

min as an antioxidant marker. However, this study is a preliminary study using a bioinformatic approach (*in silico*). Further study is still required to elucidate the mechanism *H. sabdariffa* flower as COVID-19 oral manifestation treatment with better study design and examination.

---

## CONCLUSION

---

Virtual screenings through molecular docking, an *in silico* study targeting ACE2-spike, IL-10, Foxp3, IL6, IL1B, VEGF, FGF-2, HSP70, TNFR and MDA-ovalbumin showed that anthocyanins in *H. sabdariffa* act as an antioxidant natural biomaterial has active compounds that potential as adjuvant immunoregulators therapy, which may play an important role in increasing wound regeneration of oral manifestation of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) as documented with bioinformatics exploration (*in silico*). Thus, further study is still needed to explore and elucidate the mechanism of *H. sabdariffa* active compound as a natural biomaterial for COVID-19 oral manifestations.

---

## CONFLICT OF INTEREST

---

The authors declare no conflicts of interest.

---

## ACKNOWLEDGMENTS

---

Authors would like to thank Faculty of Dental Medicine, Universitas Airlangga (Indonesia) for supporting this study (grant number: 212/UN.3/2021).

---

## REFERENCES

---

- Al-Snafi AE (2016) Pharmacological importance of *Clitoria ternatea*-A review. IOSR J Pharm 6(3): 68-83.
- Amorim Dos Santos J, Normando AGC, Carvalho da Silva RL, De Paula RM, Cembranel AC, Santos-Silva AR, Guerra ENS (2020) Oral mucosal lesions in a COVID-19 patient: New signs or secondary manifestations? Int J Infect Dis 97: 326-328.
- Arrigoni O, De Tullio MC (2002) Ascorbic acid: Much more than just an antioxidant. Biochim Biophys Acta 1569(1-3): 1-9.
- Atiqi S, Hooijberg F, Loeff FC, Rispens T, Wolbink GJ (2020) Immunogenicity of TNF-inhibitors. Front Immunol 11: 312.
- Babich O, Sukhikh S, Prosekov A, Asyakina L, Ivanova S (2020) Medicinal plants to strengthen immunity during a pandemic. Pharmaceuticals 13(10): 313.
- Bell LCK, Meydan C, Kim J, Foox J, Butler D, Mason CE, Shapira SD, Noursadeghi M, Pollara G (2021) Transcriptional response modules characterize IL-1 $\beta$  and IL-6 activity in COVID-19. iScience 24(1): 101896.
- Carvajal-Zarrabal O, Barradas-Dermitz DM, Orta-Flores Z, Hayward-Jones PM, Nolasco-Hipólito C, Aguilar-Uscanga MG, Miranda-Medina A, Bujang KB (2012)

- Hibiscus sabdariffa* L, roselle calyx, from ethnobotany to pharmacology. *J Exp Pharmacol* 4: 25–39.
- Coomes EA, Haghbayan H (2020) Interleukin-6 in Covid-19: A systematic review and meta-analysis. *Rev Med Virol* 30(6): 1–9.
- Danladi J, Sabir H (2021) Innate immunity, inflammation activation and heat-shock protein in COVID-19 pathogenesis. *J Neuroimmunol* 358: 577632.
- Dewi AMC, Dagradi EM, Wibowo P (2021) The effect of high dose vitamin C (ascorbic acid) on pro-inflammatory cytokines in COVID-19. *Med Health Sci J* 5(1): 46–50.
- Díaz Rodríguez M, Jiménez Romera A, Villarreal M (2020) Oral manifestations associated with COVID-19. *Oral Dis* DOI: [10.1111/odi.13555](https://doi.org/10.1111/odi.13555)
- Diomedea F, Marconi GD, Fonticoli L, Pizzicanella J, Merciaro I, Bramanti P, Mazzon E, Trubiani O (2020) Functional relationship between osteogenesis and angiogenesis in tissue regeneration. *Int J Mol Sci* 21(9): 3242.
- Fakeye T (2008) Toxicity and immunomodulatory activity of fractions of *Hibiscus sabdariffa* Linn (family Malvaceae) in animal models. *Afr J Tradit Complement Altern Med* 5(4): 394–398.
- Galvan-Pena S, Leon J, Chowdhary K, Michelson DA, Vijaykumar B, Yang L, Magnuson A, Manickas-Hill Z, Piechocka-Trocha A, Worrall DP, Hall KE, Ghebremichael M, Walker BD, Li JZ, Yu XG, Mathis D, Benoist C (2020) Profound Treg perturbations correlate with COVID-19 severity. *bioRxiv [Preprint]*. 12(11): 416180.
- Gani MA, Nurhan AD, Maulana S, Siswodihardjo S, Shinta DW, Khotib J (2021) Structure-based virtual screening of bioactive compounds from Indonesian medical plants against severe acute respiratory syndrome coronavirus-2. *J Adv Pharm Technol Res* 12: 120–126.
- Gollen B, Mehla J, Gupta P (2018) *Clitoria ternatea* Linn: A herb with potential pharmacological activities: Future prospects as therapeutic herbal medicine. *J Pharma Reports* 3(1): 1000141.
- Haider T, Simader E, Glück O, Ankersmit HJ, Heinz T, Hajdu S, Negrin LL (2019) Systemic release of heat-shock protein 27 and 70 following severe trauma. *Sci Rep* 9(1): 9595.
- Heck TG, Ludwig MS, Frizzo MN, Rasia-Filho AA, Homem de Bittencourt PI (2020) Suppressed anti-inflammatory heat shock response in high-risk COVID-19 patients: Lessons from basic research (inclusive bats), light on conceivable therapies. *Clin Sci (Lond)* 134(15): 1991–2017.
- Iranmanesh B, Khalili M, Amiri R, Zartab H, Aflatoonian M (2021) Oral manifestations of COVID-19 disease: A review article. *Dermatol Ther* 34(1): e14578.
- Izquierdo-Vega JA, Arteaga-Badillo DA, Sánchez-Gutiérrez M, Morales-González JA, Vargas-Mendoza N, Gómez-Aldapa CA, Castro-Rosas J, Delgado-Olivares L, Madrigal-Bujaidar E, Madrigal-Santillán E (2020) Organic acids from roselle (*Hibiscus sabdariffa* L.)—A brief review of its pharmacological effects. *Biomedicine* 8(5): 100.
- Jiang XW, Zhang Y, Zhang H, Lu K, Yang SK, Sun GL (2013) Double-blind, randomized, controlled clinical trial of the effects of diosmectite and basic fibroblast growth factor paste on the treatment of minor recurrent aphthous stomatitis. *Oral Surg Oral Med Oral Pathol Oral Radiol* 116(5): 570–575.
- Khandia R, Munjal AK, Iqbal HMN, Dhama K (2017) Heat shock proteins: Therapeutic perspectives in inflammatory disorders. *Recent Pat Inflamm Allergy Drug Discov* 10(2): 94–104.
- Kharisma VD, Ansori ANM, Widyananda MH, Utami SL, Nugraha AP (2020) Molecular simulation: The potency of conserved region on E6 HPV-16 as a binding target of black tea compounds against cervical cancer. *Biochem Cell Arch* 20(Suppl 1): 2795–2802.
- Kharisma VD, Widyananda MH, Ansori ANM, Nege A, Naw SW, Nugraha AP (2021) Tea catechin as antiviral agent via apoptosis agonist and triple inhibitor mechanism against HIV-1 infection: A bioinformatics approach. *J Pharm Pharmacogn Res* 9(4): 435–445.
- Li S, Zhang Y, Guan Z, Li H, Ye M, Chen X, Shen J, Zhou Y, Shi ZL, Zhou P, Peng K (2020) SARS-CoV-2 triggers inflammatory responses and cell death through caspase-8 activation. *Signal Transduct Target Ther* 5(1): 235.
- López-Collazo E, Avendaño-Ortiz J, Martín-Quirós A, Aguirre LA (2020) Immune response and COVID-19: A mirror image of sepsis. *Int J Biol Sci* 16(14): 2479–2489.
- Luo XH, Zhu Y, Mao J, Du RC (2021) T cell immunobiology and cytokine storm of COVID-19. *Scand J Immunol* 93(3): e12989.
- Luqman A, Kharisma VD, Ruiz RA, Götz F (2020) *In silico* and *in vitro* study of trace amines (TA) and dopamine (DOP) interaction with human alpha1-adrenergic receptor and the bacterial adrenergic receptor Qsec. *Cell Physiol Biochem* 54: 888–898.
- Marpaung A (2020) Tinjauan manfaat bunga telang (*Clitoria ternatea* L.) bagi kesehatan manusia. *J Funct Food Nutraceutical* 1(2): 47–69.
- Mehri F, Rahbar AH, Ghane ET, Soury B, Esfahani M (2021) Changes in oxidative markers in COVID-19 patients. *Arch Med Res* 52(8): 843–849.
- Mishra N, Tandon VL, Gupta R (2012) Immunomodulation by *Hibiscus rosa-sinensis*: Effect on the humoral and cellular immune response of *Mus musculus*. *Pak J Biol Sci* 15(6): 277–283.
- Plum SM, Vu HA, Mercer B, Fogler WE, Fortier AH (2004) Generation of a specific immunological response to FGF-2 does not affect wound healing or reproduction. *Immunopharmacol Immunotoxicol* 26(1): 29–41.
- Putra WE, Kharisma VD, Susanto H (2020) The exploration of medicinal plants' phytochemical compounds as potential inhibitor against human  $\alpha$ -3 nicotinic acetylcholine receptors: The insight from computational study. *AIP Conf Proc* 2231(1): 040078.
- Reang J, Sharma PC, Thakur VK, Majeed J (2021) Understanding the therapeutic potential of ascorbic

- acid in the battle to overcome cancer. *Biomolecules* 11(8): 1130.
- Sari F, Nurkhasanah, Bachri MS (2016) Acute toxicity test of roselle (*Hibiscus sabdariffa* L.) calyx ethanolic extract on Sprague Dawley rats. *Trad Med J* 21: 12–18.
- Shruthi VH, Ramachandra CT, Nidoni U, Hiregoudar S, Naik N, Kurubar AR (2016) Roselle (*Hibiscus sabdariffa* L.) as a source of natural colour: A review. *Plant Arch* 16(2): 515–522.
- Smadja DM, Philippe A, Bory O, Gendron N, Beauvais A, Gruet M, Peron N, Khider L, Guerin CL, Goudot G, Levavasseur F, Duchemin J, Pene F, Cheurfa C, Szwebel TA, Sourdeau E, Planquette B, Hauw-Berlemont C, Hermann B, Gaussem P, Samama CM, Mirault T, Terrier B, Sanchez O, Rance B, Fontenay M, Diehl JL, Chocron R (2021) Placental growth factor level in plasma predicts COVID-19 severity and in-hospital mortality. *J Thromb Haemost* 19(7): 1823–1830.
- Sokol CL, Luster AD (2015) The chemokine system in innate immunity. *Cold Spring Harb Perspect Biol* 7(5): a016303.
- Susanto H, Kharisma VD, Listyorini D, Taufiq A (2018) Effectivity of black tea polyphenol in adipogenesis related IGF-1 and its receptor pathway through *in silico* based study. *J Phys Conf Ser* 1093 (1): 012037.
- Syahrana NA, Akrom A, Darmawan E (2017) Efek serbuk bunga rosella merah (*Hibiscus sabdariffa* L.) terhadap ekspresi IL-10 pada sukarelawan sehat. *Indones J Pharm Pharm Sci* 4(1): 1–5.
- Umeoguaju FU, Ephraim-Emmanuel BC, Uba JO, Bekibele GE, Chigozie N, Orisakwe OE (2021) Immunomodulatory and mechanistic considerations of *Hibiscus sabdariffa* (HS) in dysfunctional immune responses: A systematic review. *Front Immunol* 12: 550670.
- Velavan TP, Meyer CG (2020) The COVID-19 epidemic. *Trop Med Int Health* 25(3): 278.
- Vieira AR (2021) Oral manifestations in coronavirus disease 2019 (COVID-19). *Oral Dis* 27(3): 770.
- Widyananda MH, Pratama SK, Samoedra RS, Sari FN, Kharisma VD, Ansori ANM, Yulanda A (2021) Molecular docking study of sea urchin (*Arbacia lixula*) peptides as multi-target inhibitor for non-small cell lung cancer (NSCLC) associated proteins. *J Pharm Pharmacogn Res* 9(4): 484–496
- WHO (2021) World Health Organization. <https://covid19.who.int/table> [Accessed online on: 19 March 2021]
- Yalçın B, Arda N, Tezel GG, Erman M, Alli N (2006) Expressions of vascular endothelial growth factor and CD34 in oral aphthous lesions of Behçet's disease. *Anal Quant Cytol Histol* 28(6): 303–306.
- Zhang JM, An J (2007) Cytokines, inflammation, and pain. *Int Anesthesiol Clin* 45(2): 27–37.

## AUTHOR CONTRIBUTION:

Contribution	Ramadhani NF	Nugraha AP	Rahmadhani D	Puspitaningrum MS	Rizqianti Y	Kharisma VD	Noor TNEBTA	Ridwan RD	Ernawati DS	Nugraha AP
Concepts or ideas	x	x	x	x	x	x	x	x	x	x
Design	x	x	x	x	x	x	x	x	x	x
Definition of intellectual content	x	x	x	x	x	x	x	x	x	x
Literature search	x	x	x	x	x	x	x	x	x	x
Experimental studies		x				x				
Data acquisition		x				x				
Data analysis		x				x				
Statistical analysis	x	x	x	x	x	x	x	x	x	x
Manuscript preparation	x	x	x	x	x		x	x	x	x
Manuscript editing	x	x	x	x	x		x	x	x	x
Manuscript review	x	x	x	x	x	x	x	x	x	x

**Citation Format:** Ramadhani NF, Nugraha AP, Rahmadhani D, Puspitaningrum MS, Rizqianti Y, Kharisma VD, Noor TNEBTA, Ridwan RD, Ernawati DS, Nugraha AP (2022) Anthocyanin, tartaric acid, ascorbic acid of roselle flower (*Hibiscus sabdariffa* L.) for immunomodulatory adjuvant therapy in oral manifestation coronavirus disease-19: An immunoinformatic approach. *J Pharm Pharmacogn Res* 10(3): 418–428. [https://doi.org/10.56499/jppres21.1316\\_10.3.418](https://doi.org/10.56499/jppres21.1316_10.3.418)

**Publisher's Note:** All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.