Stigmasterol, Quercetin, and Anthocyanin in Eichhornia crassipes as Host Modulation Therapy Candidate: A Bioinformatic Approach

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Abstract

Host modulation therapy (HMT) is one periodontitis treatment option for patient with risk factors that have a negative impact on the host response and oral health are difficult to maintain. Despite the potential for bacterial resistance, one of the HMTs is sub-antimicrobial-dose doxycycline. Eichhornia crassipes has anti-inflammatory, antibacterial, anti-bone resorption, and pro-osteogenesis properties. Using bioinformatics, study the active compounds stigmasterol, quercetin, and anthocyanin in E. crassipes as an HMT candidate.

Stigmasterol, quercetin, and anthocyanin were used to prepare the sample. A chemical absorption, distribution, metabolism, excretion, and toxicity (ADMET) study was performed on the compounds to assess their physicochemical characteristics, water solubility, and drug-likeness using the SWISS-ADME program. Docking simulation and molecular interaction prediction were carried out and exhibited in the form of 2D and 3D visualizations for negative binding energy. Stigmasterol, quercetin, and anthocyanin behave as drug-like chemicals with variable degrees of toxicity ranging from medium to low. Stigmasterol has the highest amount of negative binding affinity. Stigmasterol is predicted to have an inhibitory effect on nuclear factor kappa beta, tumor necrosis factor-alpha, toll-like receptor-2, matrix metalloproteinase (MMP)-1, MMP-9, tartate-resistant acid phosphatase, nuclear factor-associated T-cell-1, interleukin-1β, peptidoglycan, flagellin, and dectin, while also having the potential to modulate Interleukin-10, tissue inhibitor matrix.

Stigmasterol exhibits greater negative binding activity to antibacterial, bone remodeling, growth factor, and inflammatory cytokine indicators than quercetin, and E. crassipes anthocyanin may be a promising HMT candidate, in silico.

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Introduction

Periodontitis is a chronic inflammatory illness of the periodontal tissue that can lead to systemic problems such as innate and adaptive immune system abnormalities as well as dysbiosis of the oral cavity. Periodontitis induces

the release of pro-inflammatory mediators, such as cytokines, by leukocytes.[1] Periodontitis prevalence has been found to range from 20% to 50% worldwide.[2] Periodontitis causes a shift in polymicrobial composition, which results in a unique polymicrobial interaction function in the formation of bacterial communities in biofilms. One of which is characterized by a rise in the colonies of *Porphyromonas gingivalis* (Pg) and *Fusobacterium nucleatum* (Fn), among other species, which is connected to periodontal pocket depth and disease severity.[3] *Aggregatibacter actinomycetemcomitans (Aa) is a catalase producer that reduces hydrogen peroxide (H_2O_2), which can inhibit pathogenic Pg colonization in the oral cavity under homeostatic settings.[4] Prevotella intermedia (Pi) is an anaerobic, gram-negative bacterium that has been shown to be a pathogen in periodontal tissue injury.[5] Scaling and root planning (SRP) is now the primary line of treatment for periodontitis, however, in cases of immunocompromise, smoking habits, and other predisposing factors that are strongly associated with bacterial development, supplementary therapy is required to produce a better outcome.[6]*

Host modulation therapy (HMT) is characterized as an adjuvant therapy that improves the efficacy of scaling and root planning (SRP) by allowing the immune system to be regulated, resulting in controlled levels of cytokines, and then providing an optimal environment to initiate the healing process. HMT is intended for those who have risk factors that have a negative impact on the host response but are difficult to manage (e.g., smoking, diabetes) or cannot be modified (e.g., inherited predisposition). HMT is divided into two parts: systemically given and locally administered. Nonsteroidal anti-inflammatory medications (NSAIDs), bisphosphonates, and sub-antimicrobial-dose doxycycline (SDD) are examples of systemically given HMTs. NSAIDs, on the other hand, have the ability to alleviate gastrointestinal, renal, and hepatic issues. Bisphosphonates are widely known for their ability to cause bone necrosis and poor calcification. Furthermore, because SDD employs antibiotics, it may result in bacterial resistance. Locally administered HMTs, such as NSAIDs and enamel matrix proteins, growth factors, and bone morphogenetic proteins (BMPs), on the other hand, have different side effects, as NSAIDs are not approved by the FDA for local administration, whereas BMPs have been linked to osteolysis, neural deficits, and even cancer.[7-10] Alternative HMTs with fewer side effects are desperately needed for this disease.

Indonesia is an equatorial nation known for its abundance of water hyacinth (*Eichhornia crassipes),* also known as *eceng gondok* in Indonesian. This is a *Pontederiaceae* plant that grows on the surface of fresh water. *E. crassipes* grows swiftly and readily, threatening the aquatic ecosystems of lakes and rivers. According to study in Selorejo, Indonesia, this plant's blooms previously reached 100 out of a total of 650 hectares in the Selorejo reservoir, until fish farming in reservoirs faced crop failure due to plants taking oxygen from the waters.[11] The LD_{50} of *E. crassipes* leaves was shown to be more than 16 g/kg body weight, which is regarded as non-toxic in the short term.[12] Because this plant contains antibacterial agents such as alkaloids, flavonoids, phenols, glutathione, terpenoids, and saponins, extracts from the flowers and leaves of *E. crassipes* demonstrated significant in vitro antibacterial activity against several periodontopathogens such as Aa.[7,13]

The high amount of Stigmasterol in *E. crassipes* leaves and stems demonstrates its efficacy in preventing cancers such as ovarian, prostate, breast, and colon cancer. Furthermore, stigmasterol has been proven to suppress cholesterol production and has anti-osteoarthritic properties.[14] Stigmasterol has been shown to have analgesic, anti-inflammatory, and antioxidant properties, as well as anti-tumor potential in vivo and in vitro in several cancers via inhibition of growth and promotion of tumor cell apoptosis, increased oxidation by ROS, decreased mitochondrial, and increased Ca^{2+} concentration.[15] Stigmasterol, on the other hand, is known to affect osteogenesis in ovariectomized rats via multiple osteogenic pathways such as hypoxia-inducible factor 1 alpha (HIF-1a), mitogen-activated protein kinase (MAPK), and protein kinase B (AKT).[16]

Flavonoids are a family of chemicals that have received a lot of attention due to their antioxidant properties. Quercetin and anthocyanin are two examples of derivative chemicals. The antioxidant activity of the *E. crassipes* ethanol extract was in the very strong category, with an IC_{50} value of 48.64 mg, indicating a great capability to serve as an

antioxidant, according to study on flavonoid levels from *E. crassipes* extract fractions from Ngemplak reservoir in Indonesia.[17] The benefits of *E. crassipes* bioactive substances, it is possible to use this herbal source by mixing it with other drug carriers and expressing it in the form of gel, mouthwash, or HMT for periodontitis. The medication development procedure, which includes trials and research on animals or people, takes time and resources. A bioinformatics technique enables researchers to conduct early research using virtual simulations and analysis before moving on to more expensive and complex experimental phases. Furthermore, chemical compounds may be realistically examined on a huge scale. Researchers can concurrently analyze thousands of chemicals in search of novel pharmacological or therapeutic agents using comprehensive databases and computer analytic approaches. This enables the first identification of potential compounds prior to further testing. Prior to clinical trials, predictions of possible pharmacological activity, toxicity, and interactions with biological targets might help in the selection of more effective and safe drug candidates. The findings of this study must be validated by laboratory testing and clinical trials in humans. A bioinformatics method, on the other hand, offers the benefit of delivering early insights, speeding up the research process, and lowering the risks and costs involved with medication development.[18] Furthermore, the aim of this study is to investigate the active compounds stigmasterol, quercetin, and anthocyanin in *E. crassipe*s for HMT candidates using a bioinformatics approach, or an in-silico study.

Materials and methods

The chemical compounds of water hyacinth used in this study were stigmasterol, quercetin, and anthocyanin, so the PubChem database (https://pubchem.ncbi.nlm.nih.gov/) was used to obtain 3D molecular sample information in the structure data format (SDF), simplified molecular-input line-entry system (SMILE) canonical, and Compound ID (CID). The SDF file is minimized and converted using OpenBable v2.4.1 software to optimize the flexibility of ligand-formed atoms and convert the SDF file to the protein data bank (PDB) format. Target proteins consist of nuclear factor kappa B (NFKB), tumor-necrosing factor alpha (TNF-α), vascular endothelial growth factor (VEGF), toll like receptor-2 (TLR-2), interleukin-10 (IL-10), interleukin-1β (IL-1β), matrix metalloproteinase 1 (MMP-1), matrix metalloproteinase 9 (MMP-9), tissue inhibitor of metalloproteinases 1 (TIMP-1), translocon-associated protein (TRAP), nuclear factor of activated t cells 1 (NFATC1), collagen type I alpha 1 chain (Coll1A1), peptidoglycan, flagellin, and dectin that are assisted with the 3D structure obtained from the Research Collaborator for Structural Bioinformatics (RCSB) Protein Data Bank (PDB) database (https://www.rcsb.org/). Sterilization of the target proteins is processed using PyMol v2.5 in order to remove the contaminant molecules, including the native ligands, water, and ions, which helps in maximizing ligand-binding energy to the target protein domain.[19]

Using the SWISS-ADME (http://www.swissadme.ch/) and ProTox-II server (https://tox-new.charite.de/protoxII/), the SMILE Canonical of *E. crassipes* compounds is aided in the ADME prediction, which includes physicochemical parameters, water solubility, and drug-likeness. The goal of this procedure is to forecast the amount of toxicity based on the lethal dosage 50 (LD_{50}), similarity, and class. A drug-likeness test examination in determining the potential of a query chemical that possesses drug-like molecular properties and the capacity to reach the fixation target when the cells are undergoing metabolism. Toxicity levels are classified into six classes (I–VI). Class I and II have lethal toxicity; classes III–V have medium toxicity; and class VI has moderate toxicity. Regarding this segregation, it is strongly advised that chemicals with medium toxicity be used under particular conditions.[20]

Molecular docking can identify a ligand's potential to increase target protein activity by binding to a specific domain, which is highly dependent on the amount of binding affinity. In this study, molecular docking is performed to determine the inhibitory and modulatory actions of target proteins using a water hyacinth chemical compound. As it is considered blind docking, the PyRx v0.9.9 program is used with an auto-grid location encompassing all target protein surfaces.[21] PyMol v2.5 with cartoons, surfaces, and sticks is used to see the 3D molecular structure of the ligand-protein combination.[22,23] Using Discovery Studio v2016,

the position and kinds of chemical binding of the ligand-protein molecular complex as a result of molecular docking are then discovered. This program performs admirably when used to display the weak bonds formed by the interaction of a ligand with a protein-specific domain, since they serve critical roles in initiating the response of target proteins, including inhibition or modulation effects.[24]

No	Name	Visualization Method	PDB xID	Resolution (Å)	Weight (kDa)	Sequence Length (mer)	Chain
1	NFKB	NMR	2DBF	-	10.62	100	A
2	TNF-α	X-ray	1TNF	2.60	52.11	157	A
3	VEGF	X-ray	2VPF	1.93	95.59	102	A
4	TLR-2	X-ray	1FYX	2.80	18.36	149	A
5	IL-10	X-ray	1INR	2.00	18.67	160	A
6	IL-1β	X-ray	1HIB	2.40	17.35	153	A
7	MMP-1	X-ray	2TCL	2.20	19.63	169	A
8	MMP-9	X-ray	1L6J	2.50	47.60	425	A
9	TIMP-1	X-ray	7S7M	3.00	40.26	173	A
10	TRAP	X-ray	1WAR	2.22	35.48	310	A
11	NFATC1	NMR	1A66	-	27.33	178	A
12	CoLL1A1	NMR	2LLP	-	4.97	18	A
13	Peptidoglycan	X-ray	2OQO	2.10	23.77	200	A
14	Flagellin	X-ray	2ZBI	2.00	60.96	292	A/B
15	Dectin	X-ray	2CL8	2.80	32.92	139	A/B

Table 1. Protein target information from database.

Compounds	Physicochemical Properties	Water Solubility	Druglikeness	Toxicity
Stigmasterol	Formula: C29H48O Weight: 412.69 g/mol Num. heavy atoms: 30 Num. arom. heavy atoms: 0 Fraction Csp3: 0.86 Num. rotatable bonds: 5 Num. H-bond acceptors: 1 Num. H-bond donors: 1 Molar Refractivity: 132.75 TPSA: 20.23 Å²	Log S (ESOL): -7.46 Class: Poorly soluble Log S (Ali): -8.86 Class: Poorly soluble Log S (SILICOS-IT): -5.47 Class: Moderately soluble	Lipinski: Yes Ghose: No Veber: Yes Egan: No Muegge: No Bioavailability: 0.55	Predicted LD50: 890 mg/kg Similarity: 89.38% Predicted Toxicity Class: 4 (Medium Toxic)
Quercetin	Formula: C15H10O7 Weight: 302.24 g/mol Num. heavy atoms: 22 Num. arom. heavy atoms: 16 Fraction Csp3: 0.00 Num. rotatable bonds: 1 Num. H-bond acceptors: 7 Num. H-bond donors: 5 Molar Refractivity: 78.03 TPSA: 131.36 Å²	Log S (ESOL): -3.16 Class: Soluble Log S (Ali): -3.91 Class: Soluble Log S (SILICOS-IT): -3.24 Class: Soluble	Lipinski: Yes Ghose: Yes Veber: Yes Egan: Yes Muegge: Yes Bioavailability: 0.55	Predicted LD50: 159 mg/kg Similarity: 100% Predicted Toxicity Class: 3 (Medium Toxic)
Anthocyanin	Formula: C15H11O+ Weight: 207.25 g/mol Num. heavy atoms: 16 Num. arom. heavy atoms: 16 Fraction Csp3: 0.00 Num. rotatable bonds: 1 Num. H-bond acceptors: 1 Num. H-bond donors: 0 Molar Refractivity: 66.06 TPSA: 13.14 Å²	Log S (ESOL): -4.01 Class: Moderately soluble Log S (Ali): -3.47 Class: Soluble Log S (SILICOS-IT): -5.32 Class: Moderately soluble	Lipinski: Yes Ghose: Yes Veber: Yes Egan: Yes Muegge: No Bioavailability: 0.55	Predicted LD50: 2500 mg/kg Similarity: 71,67 % Predicted Toxicity Class: 5 (Low Toxic)

Table 2. ADMET analysis of Stigmasterol, Quercetin, and Anthocyanin.

Protein	Autogrid						Binding Affinity (kcal/mol)		
	Center (Å)			Dimensions (Å)			Stigmasterol (CID 5280794)	Quercetin (CID 5280343)	Anthocy-anin (CID 145858)
	X	Y	Z	X	Y	Z			
NFKB	42.464	14.683	38.036	90.709	67.390	51.935	-7.2	-6.7	-6.0
TNF-α	19.968	49.675	39.930	80.739	58.243	58.256	-9.8	-9.1	-7.4
VEGF	-7.420	-1.430	-4.507	52.634	48.545	39.120	-6.5	-5.5	-5.9
TLR-2	-1.855	89.786	14.769	60.692	25.608	38.363	-9.3	-8.7	-8.3
IL-10	13.024	21.379	4.401	58.672	37.623	75.759	-8.9	-6.6	-7.6
IL-1β	19.495	2.994	73.515	56.603	51.652	21.859	-8.0	-7.0	-5.9
MMP-1	63.841	5.436	16.850	57.283	39.661	44.503	-8.2	-7.2	-8.1
MMP-9	36.880	38.840	34.620	25.000	25.000	25.000	-8.2	-8.0	-7.2
TIMP-1	30.087	39.321	171.291	79.043	55.665	1.461	-9.0	-5.0	-4.6
TRAP	68.304	-24.336	17.176	25.885	27.644	39.737	-7.4	-7.2	-6.6
NFATC1	15.501	-7.918	1.696	68.461	53.410	57.713	-8.1	-8.0	-8.1
CoLL1A1	0.568	0.032	-0.246	49.934	17.523	22.589	-6.5	-6.4	-6.4
Peptido-glycan	37.648	37.735	21.932	64.278	40.527	45.926	-8.3	-8.3	-7.1
Flagellin	-23.829	37.749	33.866	149.906	40.586	98.210	-8.5	-7.6	-7.0
Dectin	43.337	20.890	45.579	55.338	39.093	31.835	-7.0	-6.6	-5.9

Table 3. Molecular docking result of Stigmasterol, Quercetin, Anthocyanin.

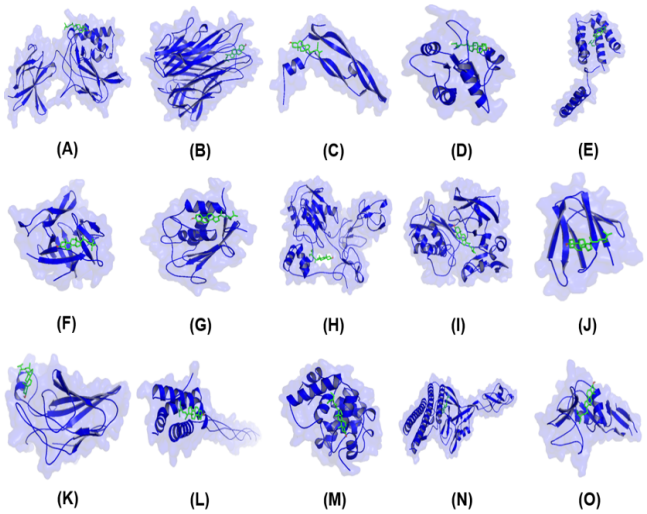


Figure 1. Visualisation of 3D molecular complex after docking simulation. (A) Stigmasterol_NFKB (B) Stigmasterol_TNF-α (C) Stigmasterol_VEGF (D) Stigmasterol_TLR-2 (E) Stigmasterol_IL-10 (F) Stigmasterol_IL-1β (G) Stigmasterol_MMP-1 (H) Stigmasterol_MMP-9 (I) Stigmasterol_TIMP-1 (J) Stigmasterol_TRAP (K) Stigmasterol_NFATC1 (L) Stigmasterol_CoLL1A1 (M) Stigmasterol_Peptidoglycan (N) Stigmasterol_Flagellin (O) Stigmasterol_Dectin.

Ligand-Protein	Chemical Interaction
Stigmasterol_NFKB	**van der Waals:** Thr146, His144, Leu143, Thr153, Arg157, Ala156, Glu63 **Pi:** Ala62, Val61, Val145, Tyr60, Lys149
Stigmasterol_TNF-α	**van der Waals:** Pro100, Gln102, Ser99, Cys101, Glu104, Arg103, Glu116 **Pi:** Cys101, Trp114, Arg103
Stigmasterol_VEGF	**van der Waals:** Met55, Cys26, Gln22, Cys102, Met55 **Pi:** Pro28, His27, Lys101, Tyr21, Tyr25
Stigmasterol_TLR-2	**van der Waals:** Asp726, Asp718, Trp712, Thr758, Thr760, Lys754, Glu727, Thr699, Asp718 **Pi:** Ile693, Phe722, Tyr715, Phe701, Ala732, Leu734, Ala731, Ile755, Lys751
Stigmasterol_IL-10	**van der Waals:** Met77, Leu65, Leu101, Leu65, Lys34 **Pi:** Arg27, Met68, Phe56, Tyr72, Ile69, Leu98, Leu94, Leu26, Phe30, Arg27
Stigmasterol_IL-1β	**Hydrogen:** Lys63 **van der Waals:** Gln38, Lys27, Asp35, Leu31, Met20, Val41, Lys65, Glu64 **Pi:** Leu29, Val19, Val40, Met36
Stigmasterol_MMP-1	**Hydrogen:** His128 **van der Waals:** Glu4, Gly5, Asn6, Gly125, Ser127, Ser129, Asp151, Gly155, Thr3 **Pi:** Ile159, Arg8, Ala158, Leu126
Stigmasterol_MMP-9	**van der Waals:** Glu47, Asp185, Gly186, Arg51, Asp182, Asn38, Met94, Gly183 **Pi:** Leu44, Leu39, Tyr52, Tyr48, Arg98, Leu187, Leu44, Leu39, Lys184
Stigmasterol_TIMP-1	**Hydrogen:** Gly71 **van der Waals:** Pro6, Glu156, Trp147, Ser161, Gln112, Leu108, Ser100, Val102, Thr98, Phe73, Glu67, His129, Tyr138, Glu156 **Pi:** Pro8, Phe101, Pro5, Leu140, Pro139, Tyr72, Ala103, Arg162, Lys157
Stigmasterol_TRAP	**Hydrogen:** His34 **van der Waals:** Ser35, Ile22, Gly23, His33, Thr52, Thr49, Gln47 **Pi:** Ala46, Leu44, Ile45, Phe9
Stigmasterol_NFATC1	**van der Waals:** Arg134, Asp130, Ile131, Leu126, Lys125, Tyr29, Glu30, Leu124 **Pi:** ALeu133, Phe78, Arg127
Stigmasterol_CoLL1A1	**van der Waals:** Ser62, Glu58, Gln55, Gly49, Glu46, Ser53, Gln42, Ile56 **Pi:** Val59, Leu67, Ala57, Ala68, Arg45
Stigmasterol_Peptidoglycan	**Hydrogen:** Lys62 **van der Waals:** Tyr65, Gln163, Asn162, Phe151, Thr150 **Pi:** Val59, Leu67, Ala57, Ala68, Arg45
Stigmasterol_Flagellin	**van der Waals:** Asp110, Lys381, Asp379, Gln176, Thr117, Ser111, Ala114, Thr382, Gln113, Asn393, Asn174, Ala411, Ser172, Asp171, Glu410, Ala409 **Pi:** Lys396, Ala412
Stigmasterol_Dectin	**van der Waals:** Trp164, Gly188, Glu120, Gln123, Gly186, Phe181, Thr185, Asn124, Arg162, Gln128, Glu132 **Pi:** His165, Phe163, Val127, Trp187

Table 4. Analysis of molecular interaction of Stigmasterol with the target proteins.

Results

Table 1 shows the protein target information from the database utilized in this investigation. Because of their physicochemical features, stigmasterol, quercetin, and anthocyanin are projected to serve as drug-like molecules in an in-silico investigation of *E. crassipes* chemical compounds. Furthermore, these compounds are projected to reach their goals through conventional pharmacological mechanisms. Because the LD_{50} for stigmasterol and quercetin is less than 1000, their toxicity level is medium, necessitating particular management. This LD_{50} level differs from anthocyanin's in that it reverts to low toxicity, as seen by such a high LD_{50} level (Table 2).

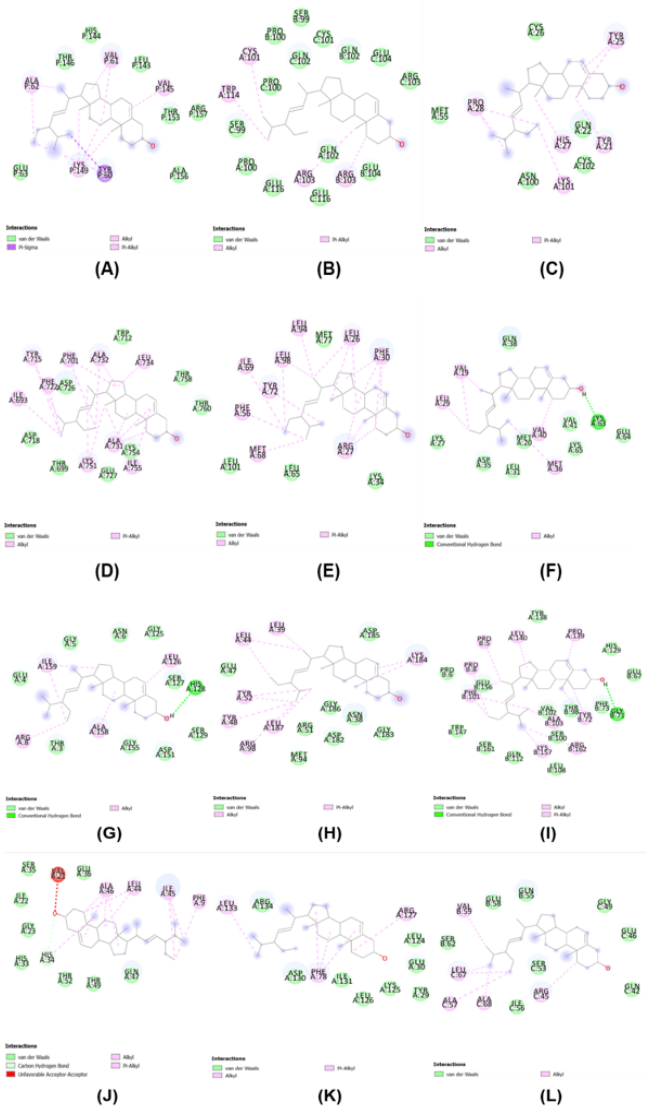
Docking simulation demonstrates that stigmasterol has a higher negative binding affinity than quercetin and anthocyanin, implying that stigmasterol will be more active in binding to the 15 target proteins (Table 3). The 3D docking simulation is visualized using PyMol v2.5 in the form of clear surfaces, cartoons, and sticks stained with a staining procedure (Figure 1). It indicates that stigmasterol inhibits NFKB, TNF-α, IL-1β, TLR-2, MMP-1, MMP-9, TRAP, NFATc1, peptidoglycan, flagellin, and dectin while also modulating IL-10, TIMP-1, CoLL1A1, and VEGF. The total binding association formed by stigmasterol may be seen in the development of stable ligand-protein complexes, which can activate activity responses on 15 target proteins, such as modulatory and inhibitory functions (Figure 2 and Table 4**).**

Discussion

Binding simulation of stigmasterol, quercetin, and anthocyanin in *E. crassipes* to the 15 target proteins with PyRx v.0.9.9. Molecular docking is a computer approach for predicting how two distinct molecules will interact to create a connection.[25,] Ligands and proteins interact to produce connections that follow two distinct principles: rigid body and induced fit. The rigid body principle states that the ligand and active site of the protein must have complementary forms, but the induced-fit principle states that if the ligand and active site of the protein do not have complementary conformations, both must undergo conformational changes to achieve fit binding. The interaction between the ligand and the protein's active site can alter and maintain protein conformational stability.[26] The affinity energy determines this connection; the lower the energy value, the greater the binding site interacts with the binding pocket, resulting in a considerable modulator or inhibiting impact on the target protein.[24] Because of the use of blind docking or screened docking, the auto-grid utilized in this study promotes contact with all regions of the protein. By targeting the whole domain of the target protein, this technique tries to acquire the ligand with the maximum negative binding activity.[23]

Identification of molecular interactions and binding positions of ligand-protein complex results show that the bonding of stigmasterol in all target proteins results in non-covalent bonding consisting of Van der Waals, π, and hydrogen binding, so the unfavorable bond interactions that are possessed by one of the molecular complexes should be not more than two weak bonds. Interactions formed by ligand binding can

initiate specific activities such as modulatory and inhibitory responses to proteins that are mediated by hydrogen bonds, hydrophobicity, van der Waals, and π.[20] Hydrogen bonds have a vital function in eliciting particular responses to target proteins and affecting medication efficacy. Water molecules interact in the docking mechanism to form hydrogen bonds, and water aids in hydrating binding spaces. The difference in energy created by the dissociation-association reaction and the interaction between water molecules and amino acids influences the free energy level. The more hydrogen bonds there are in the target protein, the greater the ligand's influence on the protein.[24] Furthermore, the interaction of stigmasterol with target proteins is known to occur via Van der Waals bonds, which occur when protons and electrons of distinct atoms combine to create energy.[23,24]



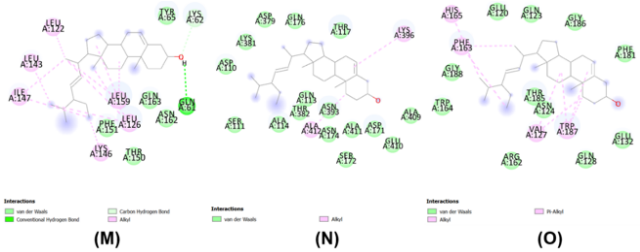


Figure 2. Visualisation of 2D position and types of chemical bonding of the complex after docking simulation. (A) Stigmasterol_NFKB (B) Stigmasterol_TNF-α (C) Stigmasterol_VEGF (D) Stigmasterol_TLR-2 (E) Stigmasterol_IL-10 (F) Stigmasterol_IL-1β (G) Stigmasterol_MMP-1 (H) Stigmasterol_MMP-9 (I) Stigmasterol_TIMP-1 (J) Stigmasterol_TRAP (K) Stigmasterol_NFATC1 (L) Stigmasterol_CoLL1A1 (M) Stigmasterol_Peptidoglycan (N) Stigmasterol_Flagellin (O) Stigmasterol_Dectin.

The hydrophobicity of proteins influences their ability to be bound to any ligand. It has a strong relationship with electric charge, and an increase in hydrophobicity is known to regulate electric charge. All protein targets contain ligand-binding sites that are either hydrophobic or hydrophilic. The hydrophobic material may attach to both hydrophobic and hydrophilic ligand-binding sites, after which it is buried inside the protein and protected from water. This situation is characterized by a stable protein-ligand interaction. Excess hydrophobicity, on the other hand, has the potential to influence ligand function by producing nonspecific binding, which decreases bond stability and makes the bond prone to rupture.[19] Binding, on the other hand, happens spontaneously when two atoms from distinct molecules meet and their shared electrons and protons are reconfigured in four directions due to polarity. Their presence strengthens the ligand-protein complex by increasing binding affinity. Unfavorable contacts are unstable bonds that form in the docking complex, and a stable ligand must have at least two of them.[23,26]

E. crassipes has three key active components that function as antibacterial agents: stigmasterol, quercetin, and anthocyanin. Flavonoid substances such as quercetin and anthocyanin have antioxidant and anti-inflammatory characteristics and can impede bacterial adherence by decreasing quorum sensing, harming plasma membranes, and

blocking nucleic acid production. Furthermore, quercetin's chemical structure, which comprises benzene, carbon, and hydroxyl groups, allows it to attach to peptidoglycan on the wall of periodontopathogenic bacteria, causing a shift in the development of the bacterial cell wall. Because both are polar, gram-negative bacteria are more vulnerable to physical attack, such as antibiotics or antibacterial compounds such as flavonoids, which may permeate the peptidoglycan wall of gram-negative bacteria. Flavonoids have been demonstrated in several trials to be beneficial against Aa at specific amounts.[20,27]

According to this study, stigmasterol, an active ingredient of *E. crassipes*, is anticipated to suppress NFKB, TNF-α, IL-1β, TLR-2, MMP-1, MMP-9, TIMP-1, TRAP, NFATc1peptidoglycan, flagellin, and dectin, as well as modify IL-10, TIMP-1, Coll1a1, and VEGF. The action of peptidoglycan and flagellin is anticipated to be inhibited by stigmasterol. Peptidoglycan is a bacterial wall compartment, whereas flagellin is a locomotory organ found mostly in gram-negative bacteria. Both are pathogen-associated molecular patterns (PAMPs) that are identified by pattern recognition receptors (PRRs), one of which is toll-like receptors (TLRs). TLRs are immune system compartments that are specifically specialized for identifying proteins from microorganisms and initiating an inflammatory cascade as a body defense strategy. Flagellin is usually identified by TLR-5 in periodontopathogenic bacteria, although peptidoglycan can be detected by TLR-2.50-52. The in-silico study predicts that stigmasterol may block TLR-2, which is confirmed by earlier research suggesting that water hyacinth can downregulate TLR5, preventing TLR-2 from sensitizing the immune system to undergo inflammatory cascades.[20]

Periodontitis interferes with the function of the downstream protein, myeloid differentiation primary response protein 88 (MyD88), preventing it from interacting with tumor necrosis factor receptor-associated factor 6 (TRAF6). This state therefore precludes the activation of transforming growth factor-activated kinase 1 (TAK-1) and transforming binding proteins 2 and 3 (TAB2/3), followed by the deactivation of I-B (IB) and mitogen-activated protein kinase (MAPK), which inhibits NF-B activation. Furthermore, stigmasterol works as an antioxidant, which increases the production of heat shock protein-70 (Hsp-70), providing a good method for downregulating the NF-kB signaling pathway. This reduces the expression of pro-inflammatory cytokines such as TNF-a and IL-1b/6. Downregulation of both proteins may suppress the production of MMP-1/9 as an enzyme that stimulates extracellular matrix (ECM) disintegration, which, when combined with stigmasterol modulation of TIMP-1, may prevent additional soft tissue degradation.[27]

TNF-a downregulation, on the other hand, reduces macrophage colony stimulating factor (M-CSF) and receptor activator of nuclear factor kappa beta ligand (RANKL) signaling. As a result, there is no absorption of the adaptor protein TRF-6, which results in suppression of NFATc-1, preventing osteoclasts from maturing. Inhibiting IL-1 lowers prostaglandin (PGE2) production, which results in the downregulation of RANK, inhibiting osteoclast differentiation, activation, and survival. Because the receptor activator of the nuclear factor kappa beta (RANK) receptor is downregulated when IL-6 is inhibited, pre-osteoclasts become less sensitive to RANKL activation.[28,29]

An in-silico study also indicates stigmasterol ability to affect VEGF, which is described as a protein that regulates angiogenesis. VEGF works by driving the production of new blood vessels to maintain oxygen and nourishment delivery to the tissue, as well as stimulating fibroblast formation and proliferation to heal injured periodontal tissue.[30,31] VEGF and FGF-2 play critical roles in osteogenesis by influencing the production of bone morphogenetic protein 2 (BMP-2) and thereby activating the suppressor of mothers against decapentaplegic 1/5/8 (Smad-1/5/8).[32,33] This is influenced by a number of osteogenic transcription factors, such as runt-related transcription factor 2 (Runx2), osterix, and alkaline phosphatase (ALP). Runx2 is a versatile transcription factor that regulates the transcription of other osteoblast-related genes, such as Coll1A1 and osteocalcin, during osteogenic development. Runx2 promotes the differentiation of mesenchymal cells into osteoprogenitor cells.[34,35] At this stage, osteoprogenitor cells proliferate into immature osteoblasts with the help of Runx 2, osterix, and distal-less homeobox 5 (Dlx5).[36,37] The presence of extracellular matrix proteins such as bone

sialoprotein, Coll1a1, and ALP aids in the transformation of immature osteoblasts into mature osteoblasts enriched in osteopontin and osteocalcin. At this moment, proteins and ALP both help the mineralization process by producing an alkaline environment in osteoid tissue that allows calcium ions to be readily deposited.[38,39] The mineralization process culminated in the development of mature bone tissue composed of osteocytes.[40,41] However, as in silico research, this drug discovery finding is restricted. More research is needed to corroborate this study outcome in an in vitro or in vivo study environment using various investigation methods.

Conclusions

As proven by a bioinformatics approach in silico, stigmasterol has more negative binding activity to antibacterial, bone remodeling, growth factor, and inflammatory cytokine biomarkers than quercetin, and anthocyanin of *E. crassipes* may be a possible candidate for HMT herbal based. Further research using various tests in vitro or in vivo is urgently needed to clarify the mechanism of *E. crassipes'* active biocompound for HMT.

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Declaration of Interest

The authors report no conflict of interest.

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