Molecular Docking of Marumoside, Rutin, and Quercetin in Moringa oleifera to Bone Remodeling Biomarkers: An in-silico study

Ari Triwardhani[1], Alexander Patera Nugraha[1]*, Leonardi Hadianto[1], Diona Olivia Yudianto[1], Dina Kumala[1], Fransisca Shancti Rosari[1], Ida Bagus Narmada[1], I Gusti Aju Wahju Ardani[1], Ratri Maya Sitalaksmi[2], Nastiti Faradilla Ramadhani[3], Muhammad Luthfi[4], Viol Dhea Kharisma[5], Albertus Putera Nugraha[6], Florentina Joestandari[7], Tengku Natasha Eleena binti Tengku Ahmad Noor[8,9]

1. Orthodontics Department, Faculty of Dental Medicine, Universitas Airlangga, Surabaya, Indonesia.
2. Prosthodontics Department, Faculty of Dental Medicine, Universitas Airlangga, Surabaya, Indonesia.
3. Dentomaxillofacial Radiology Department, Faculty of Dental Medicine, Universitas Airlangga, Surabaya, Indonesia.
4. Oral Biology Department, Faculty of Dental Medicine, Universitas Airlangga, Surabaya, Indonesia.
5. Biology, Faculty of Science and Technology, Universitas Airlangga, Surabaya, Indonesia.
6. Faculty of Medicine, Universitas Airlangga, Surabaya, Indonesia.
7. Faculty of Dentistry, Bhakti Wiyata Health Institute, Kediri, Indonesia.
8. Membership of Faculty of Dental Surgery, Royal Collage of Surgeon, Edinburgh University, United Kingdom.
9. Military Dental Officer of Royal Medical and Dental Corps, Malaysian Armed Forces, Semenggo Camp, Kuching, Serawak, Malaysia.

Abstract

Marumoside, Rutin and Quercetin are Moringa oleifera (MO) bioactive compounds which may act as anti-oxidant, anti-bacterial, anti-bone resorption, anti-inflammatory agent, pro-osteogenic and pro-growth factor that beneficial during bone remodeling.

Aim: to analyze the Marumoside, Rutin and Quercetin of MO to Tartate Resistant Acid Phosphatase (TRAP), Nuclear Factor T-Cell (NFATc1), Nuclear Factor Kappa Beta (NFKB), Tumor Necrosis Factor Alpha (TNF-a), Heat Shock Protein (HSP)-70, HSP-10, receptor activator nuclear kappa beta and its ligand (RANK-RANKL), matrix metalloproteinase-9 (MMP-9), Peptidoglycan, Flagellin, Dectin, Runt Related Transcription Factor-2 (RUNX2), Osterix, Osteoprogetrin (OPG), Vascular Endothelial Growth Factor (VEGF), fibroblast growth factor-2 (FGF-2), collagen type 1 alpha-1 Coll1a1) through bioinformatics approach, an in silico study.

Chemical compounds from MO used in this study consisted of Marumoside, Rutin and Quercetin obtained from PubChem database. The target proteins with 3D structures obtained from RCSB-PDB database. Canonical SMILE from MO compound was used to predict absorption, distribution, metabolism, excretion, toxicity (ADMET) consisting of physicochemical properties, water solubility, and drug-likeness, toxicity level based on LD50.

Marumoside, rutin, and quercetin of MON act as drug-like molecules and toxicity were low. Quercetin has greatest negative binding energy and it was predicted to inhibit TRAP, NFATc1, NFKB, TNF-a, HSP-70, RANK, MMP-9, Peptidoglycan, Flagellin, Dectin and upregulate RUNX2, Osterix, HSP-10, RANKL, OPG, VEGF, FGF-2, Coll1a1 in silico. Conclusion Quercetin of MO is predicted to trigger inhibition activity of bone resorption, pro-inflammatory cytokines as well as triggering the increased activity of bone apposition, antioxidant, anti-inflammatory cytokine and growth factor.

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Corresponding author:
Alexander Patera Nugraha, DDS., MSc., MDSc., Ph.D.,
Faculty of Dental Medicine Universitas Airlangga Indonesia. Jl. Mayjen Prof. Dr. Moestopo No.47, Surabaya, East Java 60132 Indonesia.
E-mail: alexander.patera.nugraha@fkg.unair.ac.id

Introduction

The Moringa genus is a subtropical tree native to Asia and Africa that comprises 13 species, the most cultivated of which is *Moringa oleifera Lam.* (MO).[1] MO is a perennial deciduous tropical tree endemic to northern India's Himalayan Mountains. It belongs to the

Moringaceae family. MO has a high concentration of proteins, vitamin A, minerals, essential amino acids, antioxidants, flavonoids, and isothiocyanates.[2] MO is also known as the "miracle plant" since it has long been utilized as a food source and medication to heal a variety of maladies.[1] MO's positive effects are largely linked to its phytochemicals, such as flavonoids and isothiocyanates, which have bioactivity.[2] MO leaves, seeds, bark, roots, sap, and flowers are commonly employed in traditional medicine, while the leaves and immature seed pods are used as food. MO Leaf extracts have the highest antioxidant activity, and numerous animal safety studies employing aqueous leaf extracts show a high level of safety. There have been no reports of harmful effects associated with human study. Five human investigations utilizing powdered whole leaf preparations of MO have shown anti-hyperglycemic and anti-dyslipidemic activity. In animal investigations, these actions were validated using MO extracts as well as MO leaf powders. Aqueous, hydroalcohol, or alcohol extracts of MO leaves have a variety of additional biological activities, including antioxidant, tissue protective, analgesic, antiulcer, antihypertensive, radioprotective, and immunomodulatory properties.[3]

MO, often known as a tree of life, has been widely used as a functional food and nutritional asset all over the world. MO's ethnomedicinal and traditional applications suggest that it may have pleiotropic therapeutic activity against the majority of human diseases. In fact, MO has been shown to have antioxidant, antibacterial, antifungal, antipyretic, antiulcer, anticancer, hepatoprotective, and heart stimulating effects.[4] Diverse research has been conducted to extract and examine the activities of its diverse bioactive components, including polysaccharides, due to the varied biological activities of different sections of MO. polysaccharides of MO have been shown to contain a range of biofunctionalities. Aside from bioactive polysaccharides, the gum exuded by the stem of this plant is commercially important, with several uses in the pharmaceutical industry. Polysaccharides of MO have been isolated and purified using a variety of extraction and purification techniques, as well as a combination of approaches. According to studies, extraction procedures affect the structure of polysaccharides and consequently their biological activity.[5]

MO contains rutin, gallic acid, and several other bioactive chemicals, all of which contribute to its shown spectrum of pharmacological properties.[6] Rutin was one of the most often reported bioactive substances, which all belong to the polyphenolic compound family. Rutin may be a useful active ingredient for increasing insulin production, immunity, and/or decreasing blood glucose levels in Type 2 Diabetes Mellitus (T2DM).[7] MO is an excellent traditional medication for treating inflammatory diseases. Previously, three active chemicals in MO were isolated and identified, namely niazirin, marumoside A, and sitosterol-3-O--d-glucoside. MO active substances inhibited the expression of Interleukin (IL)-12/IL-23 phenotype(p)40, IL-17A, IL-22, and IL-23 p19 in vitro.[8]

MO significantly reduced inflammation response and alveolar bone resorption in a periodontitis animal model. The anti-periodontitis effect was attributed to antioxidant capabilities of MO. Hematoxylin eosin (H&E) staining of mice's kidney, heart and the survival rate of osteoclast cell line cells were used to confirm biocompability of MO. Finally, MO appears to be a safe way to prevent chronic periodontitis by modulating the expression of p38 mitogen-activated protein kinase (p38-MAPK)-Osteoprotegrin(OPG)/receptor activator nuclear kappa beta ligand (RANKL).[9] MO are also calcium-rich candidates for creating effective calcium supplements. Microbial fermentation boosted MO calcium bioavailability, promoted the growth and development of calcium-deficient animal models, bone calcium deposition, and bone growth. Thus, MO improved bone strength; reduced bone resorption; and prevented calcium deficit.[10] Previous research shown that combining MO extract with demineralized freeze-dried bovine bone xenograft (DFDBBX) successfully increased TGF-1 and osteocalcin expression for alveolar bone repair in the tooth extraction socket animal model.[11] MO leaves are high in bioactive chemicals that can aid in the treatment of obesity-related illnesses like T2DM. MO supplementation at 280 mg/kg BW/day promotes brown adipose tissue (BAT) development and proliferation in an animal model fed a high fat diet (HFD) through increasing Bone Morphogenetic Protein (BMP)-7 protein levels.[12]

MO includes a high concentration of complex nutrients as well as an active

component with anti-osteoporosis properties. MO has anti-osteoporotic effectiveness, which might be mediated through gut microbiota modulation and MAPK signaling.[13] By stimulating the PI3K/Akt/Foxo1 pathway, MO leaf extract reduces peroxidative damage and increases osteogenic induction of rat Bone Marrow Mesenchymal Stem Cell (BMSCs).[14] Previous research also shown that a titanium disc covered with MO hydrogel and seeded with Human Mesenchymal Stem Cells (MSCs) increases osteoblast cell growth.[15] In addition, MO floral extract significantly increased the growth of rat fibroblasts and MSCs. The extract was also shown to be angiogenic and hepatoprotective. The extract had no effect on cancer cell line growth, indicating that it is safe for human consumption and usage in medicines. leaf extract of MO demonstrated a low capacity for cell stimulation but a strong cytotoxic impact on cancer cell lines.[16] Myricetin, quercetin, and kaempferol are the major flavonoids contained in MO leaves. MO tree parts are utilized for a range of nutritional and therapeutic applications. Quercetin, phenolic acid, tannins, and saponins have been linked to MO's hypolipidemic, antihypertensive, antioxidant, anti-cancer, anti-diabetic, and hepatoprotective activities.[17]

The hypothesis of this study is Marumoside, Rutin, Quercetin as bioactive of MO may act as anti-oxidant, anti-bacterial, anti-bone resorption, anti-inflammatory agent, pro-osteogenic and pro-growth factor that may beneficial during bone remodeling. Thus, the aim of this study is to analyze the molecular docking of Marumoside, Rutin and Quercetin of MO to Tartate Resistant Acid Phosphatase (TRAP), Nuclear Factor T-Cell (NFATc1), Nuclear Factor Kappa Beta (NFKB), Tumor Necrosis Factor Alpha (TNF-a), Heat Shock Protein (HSP)-70, HSP-10, receptor activator nuclear kappa beta and its ligand (RANK-RANKL), matrix metalloproteinase-9 (MMP-9), Peptidoglycan, Flagellin, Dectin, Runt Related Transcription Factor-2 (RUNX2), Osterix, Osteoprogetrin (OPG), Vascular Endothelial Growth Factor (VEGF), fibroblast growth factor-2 (FGF-2), collagen type 1 alpha-1 Coll1a1) through bioinfomartic approach, an in silico study.

Materials and methods

Chemical compounds from MO used in this study consisted of marumoside, rutin, and quercetin, then information on 3D compound samples with file structure data format (SDF), Canonical SMILE, and CID obtained from PubChem database.[18] Minimizing and converting process of structure data format (SDF) files is done through OpenBable v2.4.1 software.[19] The target proteins consist of nuclear factor kappa beta (NF-κβ), tumor necrosis factor alpha (TNF-α), runt related transcription factor-2 (RUNX2), Osterix, nuclear factor associated T-cells 1 (NFATc1), tartate resistance acid phosphatase (TRAP), heat shock protein-10 (HSP-10), HSP-70, vascular endothelial growth factor (VEGF), fibroblast growth factor-2 (FGF-2), matrix metalloproteinase-9 (MMP-9), collagen type 1A1 (COLL1A1), peptidoglycan, flagellin, and dectin with 3D structures obtained from RCSB-PDB database.[20,21] The sterilization process is done with PyMol v2.5 software for contaminant molecules such as native ligands, water, and ions. Canonical SMILE from MO compound was used to predict ADME consisting of physicochemical properties, water solubility, and drug-likeness through the SWISS-ADME server and the ProTox-II server to predict toxicity level based on LD50, similarity, and class. PyRx v0.9.9 software is used with autogrid position. Visualization of the 3D structure of ligand-protein complex was carried out using PyMol v2.5 software then the chemical bond interaction type and position is identified by Discovery Studio v2016 software.[22-24]

Results

Information from the database consisting of name, visualization method, PDB ID, resolution, weight, sequence length, and chain of fifteen target proteins has been obtained (Table 1), based on ADME predictions that marumoside, rutin, and quercetine compounds are predicted to be drugs- like molecule because it fulfills most of the druglikeness parameters, then the three compounds can be soluble and, it is predicted to reach targets such as drug mechanisms in general, then the toxicity level of marumoside and rutin compounds is low because they have a high LD50 value, quercetin toxicity is classified as medium because the LD50 value is < 1000, it shows that the use of compounds with medium toxicity must undergo special handling (Table 2). The docking results show that Quercetin has a

more negative binding affinity value than Rutin & Marumoside, Quercetin is predicted to have higher activity when it binds to the fifteen target proteins (Table 3). Identification of molecular interactions and binding positions on the docked protein-ligand complex showed that the bonding of Quercetin compounds on the entire target protein resulted in non-covalent bond interactions consisting of Van der Waals, pi, and hydrogen, then unfavorable bond interactions possessed by one of the molecular complexes that is not more than two (Table 4).

No	Name	Visualization Method	PDB ID	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	RUNX2	X-ray	6VGE	4.25	62.53	117	D
4	OSTERIX	NMR	6X46	-	14.35	121	A
5	NFATC1	NMR	1A66	-	27.33	178	A
6	TRAP	X-ray	1WAR	2.22	35.48	310	A
7	HSP-10	NMR	6MRD	3.82	27.33	178	C
8	HSP-70	X-ray	1S3X	1.84	42.75	382	A
9	VEGF	X-ray	2VPF	1.93	95.59	102	A
10	FGF-2	NMR	1BLA	-	17.35	155	A
11	MMP-9	X-ray	1L6J	2.50	47.60	425	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
Marumoside	Formula: C14H19NO6 Weight: 297.30 g/mol Num. heavy atoms: 21 Num. arom. heavy atoms: 6 Fraction Csp3: 0.50 Num. rotatable bonds: 4 Num. H-bond acceptors: 6 Num. H-bond donors: 4 Molar Refractivity: 72.11 TPSA: 122.24 Å²	Log S (ESOL): -0.87 Class: Very soluble Log S (Ali): -0.86 Class: Very soluble Log S (SILICOS-IT): -0.76 Class: Soluble	Lipinski: Yes Ghose: No Veber: Yes Egan: Yes Muegge: Yes Bioavailability: 0.55	Predicted LD50: 4000 mg/kg Similarity: 77.21% Predicted Toxicity Class: 5 (Low Toxic)
Rutin	Formula: C27H30O16 Weight: 610.52 g/mol Num. heavy atoms: 43 Num. arom. heavy atoms: 16 Fraction Csp3: 0.44 Num. rotatable bonds: 6 Num. H-bond acceptors: 16 Num. H-bond donors: 10 Molar Refractivity: 141.38 TPSA: 269.43 Å²	Log S (ESOL): -3.30 Class: Soluble Log S (Ali): -4.87 Class: Moderately soluble Log S (SILICOS-IT): -0.29 Class: Soluble	Lipinski: Yes Ghose: No Veber: No Egan: No Muegge: No Bioavailability: 0.17	Predicted LD50: 5000 mg/kg Similarity: 100% Predicted Toxicity Class: 5 (Low 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)

Table 2. ADMET analysis of marumoside, rutin, and quercetin of MO.

The overall weak binding interaction generated by Quercetin can contribute to the formation of stable ligand-protein complexes and initiate activity responses on target proteins such as enhancement and inhibition. Marumoside, rutin, and quercetin of MON act as drug-like molecules and toxicity was low. Quercetin was predicted to inhibit TRAP, NFATc1, NF-κβ, TNF-α, HSP-70, RANK, MMP-9, Peptidoglycan, Flagellin, Dectin and upregulate RUNX2, Osterix, HSP-10, RANKL, OPG, VEGF, FGF-2, COLL1A1 in silico (Figure 1 and Figure 2).

Protein	Autogrid						Binding Affinity (kcal/mol)		
	Center (Å)			Dimensions (Å)					
	X	Y	Z	X	Y	Z	Marumoside	Rutin	Quercetin
NFKB	-13.074	8.026	-26.463	38.151	42.830	73.905	-6.1	-7.7	-7.8
TNF-α	8.075	25.301	22.863	31.622	16.932	12.741	-4.2	-5.0	-9.0
RUNX2	38.335	17.716	19.753	88.374	72.496	93.243	-6.8	-8.5	-8.5
OSTERIX	-18.049	-21.892	-40.965	35.286	56.805	38.239	-5.8	-7.0	-7.1
NFATC1	21.772	9.256	9.271	39.081	45.617	55.099	-5.9	-6.7	-7.7
TRAP	79.198	35.787	45.693	54.126	36.155	43.192	-6.1	-7.2	-7.2
HSP-10	43.872	23.205	9.204	64.911	70.704	66.173	-6.8	-8.1	-8.2
HSP-70	-50.95	39.75	-15.61	25.00	25.00	25.00	-5.6	-6.2	-7.6
VEGF	40.018	29.476	52.605	54.253	99.095	80.499	-6.6	-8.2	-8.5
FGF-2	3.937	3.965	-46.055	66.285	36.739	27.656	-5.6	-7.2	-8.1
MMP-9	68.142	-24.322	17.856	33.318	38.191	40.670	-6.3	-7.2	-7.9
COLL1A1	0.568	0.032	-0.246	49.934	17.523	22.589	-4.6	-5.3	-6.4
Peptidoglycan	37.648	37.735	21.932	64.278	40.527	45.926	-7.4	-8.9	-8.9
Flagellin	-23.829	37.749	33.866	149.906	40.586	98.210	-6.4	-8.8	-8.9
Dectin	43.337	20.890	45.579	55.338	39.093	31.835	-6.4	-7.4	-8.0

Table 3. The docking result of marumoside, rutin, and quercetin to molecular targets.

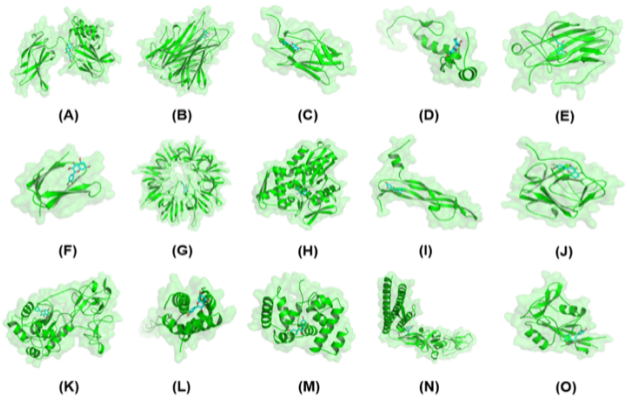

Figure 1. Three-dimension visualization complex molecule from the result of molecular docking simulation. (A) Quercetin_NFKB; (B) Quercetin_TNF-α; (C) Quercetin_RUNX2; (D) Quercetin_Osterix; (E) Quercetin_NFATc1; (F) Quercetin_TRAP; (G) Quercetin_HSP-10; (H) Quercetin_HSP-70; (I) Quercetin_VEGF; (J) Quercetin_FGF-2; (K) Quercetin_MMP-9; (L) Quercetin_COLL1A1; (M) Quercetin_Peptidoglycan; (N) Quercetin_Flagellin;(O) Quercetin_Dectin.

Ligan-Protein	Chemical Interaction
Quercetin_NFKB	**Hydrogen:** Lys52, Gly55, Gly69, Lys79, Ser74 **van der Waals:** Ser75, Ser81, Gly72, Gly68, Phe56 **Pi:** Lys80, Pro71 **Unfavorable:** Lys52
Quercetin_TNF-α	**Hydrogen:** Glu116, Gln102, Arg103, Ser99 **van der Waals:** Pro117, Lys98, Tyr115, Pro100, Pro101, Glu116, Gln102, Cys101, Glu104 **Pi:** Glu166 **Unfavorable**: Glu166, Gln102
Quercetin_RUNX2	**Hydrogen:** Arg215, Tyr213 **van der Waals:** Ser196, Lys218, Val114, Val125, Leu126, Ala216, Pro127, Ser128, Ile217, His129
Quercetin_OSTERIX	**Hydrogen:** Ser75, Asp80, Glu78, His57 **van der Waals:** Lys74, Asn54, Arg56, Gln79, Ile77, Ser76 **Pi:** Asp80
Quercetin_NFATC1	**Hydrogen:** Val 81, Gln80. His25 **van der Waals:** Pro21, Ser169, Ile118, Phe151, Gly122, Lys102, Tyr79, Ile65 **Pi:** Leu149, Ile172, Pro46, Ala121, Leu104
Quercetin_TRAP	**Hydrogen:** Gly23, Gln47, Val21, Thr49 **van der Waals:** His51, His33, Thr52, Glu36, Ile22, Lys56 **Pi:** Ile55, Ala46
Quercetin_HSP-10	**Hydrogen:** Asp96, Phe55, Asp57 **van der Waals:** Leu14, Gln66, Pro65, Asp16, Gly54, Thr56, Ala58 **Pi:** Val63, Phe55
Quercetin_HSP-70	**Hydrogen:** Tyr41 **van der Waals:** Arg272, Glu268, Tyr15, Lys56, Thr14, Gly203, Arg72, His227, Trp90, Phe68, Arg264, Gly202 **Pi:** Asp69, Glu231
Quercetin_VEGF	**Hydrogen:** Lys107, Cys57, Asp63, Cys61 **van der Waals:** Gly58, Gly59, Glu64, Asn62, Leu66, Cys60, Glu67, Val69 **Pi:** Cys68 **Unfavorable:** Lys107
Quercetin_FGF-2	**Hydrogen:** Ala11, Ser155, Thr8 **van der Waals:** Leu12, Leu149, Pro10, Leu9, Arg31, Ala153, Thr7, Ile6, Ser152 **Pi:** Pro13, Met151, Thr9
Quercetin_MMP-9	**Hydrogen:** Arg95, Asn38 **van der Waals:** Arg51, Leu44, Asp185, Pro97, Thr96, Met94, Gly186, Arg98, Tyr52, Leu187, Tyr48, Glu47 **Pi:** Asp182
Quercetin_COLL1A1	**Hydrogen:** Pro66 **van der Waals:** Lys65, Glu66, Val59 **Pi:** Ser62, Ala63, Leu67 **Unfavorable:** Ala68
Quercetin_Peptidoglycan	**Hydrogen:** Gln113, Asp94, Asn86 **van der Waals:** Arg225, Arg218, Lys201, Thr82, Gly114, Glu83, Ser116, Gly115, Thr117 **Pi:** Arg100, Asp84 **Unfavorable:** Lys85
Quercetin_Flagellin	**Hydrogen:** Leu173, Asn174, Ala412, Val378, Lys396 **van der Waals:** Ser165, Leu120, Asp171, Ser172, Ala413, Gln113, Ala411, Gly377, Asp379, Thr382, Gln176, Asn393 **Pi:** Thr117
Quercetin_Dectin	**Hydrogen:** Ser88, Asn205 **van der Waals:** Thr119, Asn118, Glu203, Val115, Leu114, Ile152, His113, Ser89 **Pi:** Asp153, Val166

Table 4. Molecular analysis results on Quercetin compound with target protein domain.

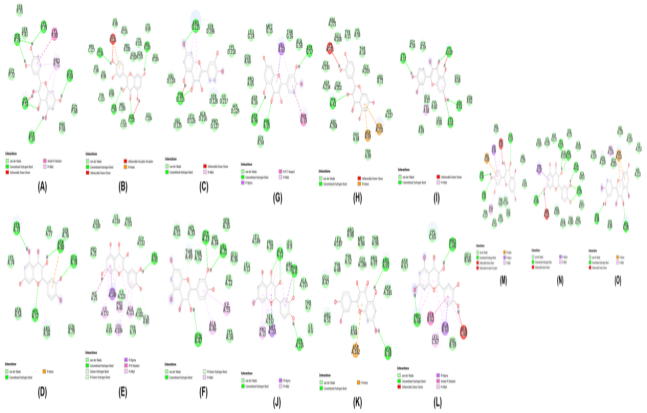

Figure 2. Two-dimension visualization of location, type of chemical bond to the molecular complex from the result of molecular docking simulation. (A) Quercetin_NFKB; (B) Quercetin_TNF-α; (C) Quercetin_RUNX2; (D) Quercetin_Osterix; (E) Quercetin_NFATc1; (F) Quercetin_TRAP; (G) Quercetin_HSP-10; (H) Quercetin_HSP-70; (I) Quercetin_VEGF; (J) Quercetin_FGF-2; (K) Quercetin_MMP-9; (L) Quercetin_COLL1A1; (M) Quercetin_Peptidoglycan; (N) Quercetin_Flagellin;(O) Quercetin_Dectin.

Discussion

In silico study of MO reveal that Marumoside, Rutin, and Quercetin compounds are predicted to be drug-like molecules based on the physicochemical properties and solubility. Marumoside and rutin have low toxicity with LD50 exceeding 1000, while quercetin has medium toxicity because the LD_{50} is below 1000 thus requires special handling.[25] Molecular docking simulation with autogrid shows that quercetin has the most negative binding energy that strengthens binding with 15 target proteins to exert significant modulating or inhibitory function.[26,27] Quercetin is predicted to trigger inhibition of TRAP proteins, NFATc1, NF-κβ, TNF-α, HSP-70, RANK, MMP-9, Peptidoglycan, Flagellin, Dectin as well as triggering the increased activity of RUNX2, Osterix, HSP-10, RANKL, OPG, VEGF, FGF-2, Coll1A1.

Quercetin binds to target proteins by undergoing several non-covalent bindings including Van der Waals, π, and hydrogen binding and unfavorable binding either donor-donor or acceptor-acceptor interaction that is observed not more than 2 in one molecular complex. All bond discovered were manifest in upregulation of binding energy due to shortening

of distance between different atoms and sharing of electrons between quercetin and target proteins resulting in stable binding.[28-30] Utilization of MO extract may able to prevent downstream pathways of toll like receptor (TLRs) in recognizing pathogen associated molecule pattern (PAMP) (including LPS, flagellin, and peptidoglycan). Recognition of PAMP by TLRs leading to activation of MyD88 and TAK1 thus induces IKKα and IKKβ in order to upregulate NF-κβ expression.[31-33]

MO contains vitamin C and vitamin E derivatives (α-tocopherol, β-sitosterol) as antioxidants and is able to upregulate HSP70 as their presence is able to inhibit activation of NF-κβ as an inflammatory pathway. This condition is also supported by MO ability to induce IL-10 expression. Downregulation of NF-κβ manifests in inhibition of MMP-9 by modulating TIMP-1. This condition leads to stability of ECM thus preventing further soft tissue destruction.[34-37] Inhibition of NF-κβ will reduce TNF-α, Interleukin (IL) IL-1β, and IL-6 with high serum levels in inflammation conditions.[38,39] Downregulation of TNF-α expression occurs due to a direct decrease in M-CSF expression and lack of RANKL signaling.[40-42] This condition results in no uptake of the adapter protein TRF-6 which manifests as inhibition of NFATc-1 thus preventing $TRAP^{+}$ osteoclast to generate maturation.[43,44] Inhibition of IL-1β reduces prostaglandin E2 (PGE2) synthesis resulting in the downregulation of RANK thus preventing differentiation, activation, and survival of osteoclast cells. Inhibition of IL-6 decreases pre-osteoclasts sensitivity to RANKL stimulation due to the downregulation of the RANK receptor.[45] In addition, inhibition of IL-6 prevents the promotion of Parathyroid hormone-related protein (PTHrP) secretion through TNF-α which will directly reduce osteoclast activity significantly and prevent osteolysis. Downregulation of IL-6 will increase osteoblast differentiation through increased expression of ALP, RUNX2, and osteocalcin genes.[46,47]

MO with potential antioxidant ability may induce the expression of several osteogenic proteins including RUNX2, alkaline phosphatase (ALP), osterix (OSX), osteocalcin (OCN), and collagen type 1a1 (coll1a1).[48] RUNX2 is an important multifunctional transcription factor for the osteogenic markers during osteogenic differentiation and it can control the transcription of other osteoblast-related genes like COL1A1 and OCN. Pre-osteoblast cells that express RUNX2 will develop into mature osteoblasts that express the genes for late osteogenic differentiation.[49]

A variety of extracellular matrix proteins, including OCN, bone sialoprotein, and osteopontin, are also produced by mature osteoblasts. OCN is widely regarded as an important marker for osteoblasts. RUNX2 and OSX both function downstream of one another and are expressed in pre-osteoblast.[50] RUNX2 and OSX are the two fundamental transcription elements that boost osteoblastic differentiation. OCN can also be used as a biomarker in accurately evaluating the bone formation process. The rising ALP enzyme in cultivated osteoblast cells can be used to determine the osteogenic effects of flavonoids. ALP is involved in the mineralization process and works to produce an alkaline environment in the newly created osteoid tissue so that calcium can be deposition in the tissue is simple. Specific ALP, meantime, contributes to the transformation of bone into osteoblasts and reflects osteoblast cell activity during bone development.[51] The expression of Coll1a1, a protein that is highly and specifically expressed in cartilage, increased in late-stage chondrogenesis, supporting the differentiation process.[52] Additionally, MO is known to increase the expression of VEGF and FGF2. One of the main proteinases and a leading candidate for promoting angiogenesis through the activation and expansion of endothelial cells, macrophages, and blood vessels has been identified as VEGF.[53,54] FGF-2 stimulates fibroblast formation and proliferation to repair damaged tissue and form complex adhesion during the bone remodeling.[51,54,55]

Conclusions

According to this research, Quercetin of MO is predicted to trigger inhibition activity of bone resorption, pro-inflammatory cytokines, virulence factor as well as triggering the increased activity of bone apposition, antioxidant, anti-inflammatory cytokine and growth factor.

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Declaration of Interest

The authors should declare if exist or not conflict of interest with the data contained in the manuscript.

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