

# In Silico Interaction of the Active Compounds of *Scurrula Atropurpurea* with the RANK/RANKL/OPG System in Diabetoporosis

Izaak Zoelkarnain Akbar<sup>1,4</sup>,  
Firli Rahmah Primula Dewi<sup>2</sup>,  
Bambang Setiawan<sup>3,4</sup>

<sup>1</sup>Department of Orthopaedics and Traumatology, Ulin General Hospital, Faculty of Medicine, Universitas Lambung Mangkurat, Banjarmasin, South Kalimantan, Indonesia

<sup>2</sup>Malang In Silico Club, Malang, East Java, Indonesia

<sup>3</sup>Department of Biochemistry and Molecular Biology, Faculty of Medicine, Universitas Lambung Mangkurat, Banjarmasin, South Kalimantan, Indonesia

<sup>4</sup>Research Center for Osteoporosis, Faculty of Medicine, Universitas Lambung Mangkurat, Banjarmasin, South Kalimantan, Indonesia

Corresponding author: Izaak Zoelkarnain Akbar, MD., PhD, Department of Orthopaedics and Traumatology, Ulin General Hospital, Faculty of Medicine, Universitas Lambung Mangkurat, Banjarmasin, South Kalimantan, Indonesia, Email: [izaakakbar@yahoo.co.id](mailto:izaakakbar@yahoo.co.id)

doi: 10.5455/aim.2019.27.8-11

ACTA INFORM MED. 2019 MAR 27(1): 8-11

Received: Jan 05, 2019 • Accepted: Mar 05, 2019

© 2019 Izaak Zoelkarnain Akbar, Firli Rahmah Primula Dewi, Bambang Setiawan

This is an Open Access article distributed under the terms of the Creative Commons Attribution Non-Commercial License (<http://creativecommons.org/licenses/by-nc/4.0/>) which permits unrestricted non-commercial use, distribution, and reproduction in any medium, provided the original work is properly cited.

## ABSTRACT

**Introduction:** Diabetoporosis is a very complex health problem in Indonesia. One approach to the problem is through native Indonesian herbal medicine. The application of *Scurrula atropurpurea* in the treatment of diabetoporosis has not been revealed, so preliminary *in silico* study needs to be done. **Aim:** The purpose of the present study was to analyze the interaction between the active compound of *Scurrula atropurpurea* and the RANK/RANKL/OPG system in the pathomechanism of osteoporosis in diabetes mellitus. **Methods:** The procedures of the study included the search for the constituent amino acid of the RANK/RANKL/OPG system, the search for the structure of the active component of *Scurrula atropurpurea*, 3D modeling of protein structure, protein-ligand docking and visualization, and analysis of protein-ligand bonding interactions. **Results:** Those bond energies were RANKL-aviculin (-274.96 kJ/mol), RANKL-rutin (-263.12 kJ/mol), RANKL-quercitrin (-256.98 kJ/mol), RANKL-quercetin (-226,50 kJ/mol), RANKL-kaempferol (-221,65 kJ/mol), RANKL-catechin (-214,85 kJ/mol), RANKL-epicatechin (-211.66 kJ/mol), RANKL-caffeine (-171.73 kJ/mol), and RANKL-theobromine (-161.14 kJ/mol). The bond energies were RANK-rutin (-719.26 kJ/mol), RANK-catechin (-680.15 kJ/mol), RANK-caffeine (-654.48 kJ/mol), RANK-theobromine (-651.77 kJ/mol), RANK-quercitrin (-650.68 kJ/mol), RANK-kaempferol (-643.03 kJ/mol), RANK-epicatechin (-641.86 kJ/mol), RANK-quercetin (-641.76 kJ/mol), and RANK-aviculin (-628.62 kJ/mol). Those bond energies were OPG-epicatechin (-590.09 kJ/mol), OPG-theobromine (-578.08 kJ/mol), OPG-caffeine (-568.88 kJ/mol), RANKL-catechin (-560.63 kJ/mol), OPG-quercitrin (-554.50 kJ/mol), OPG-rutin (-547.91 kJ/mol), OPG-quercetin (-545.75 kJ/mol), OPG-kaempferol (-544.48 kJ/mol), and OPG-aviculin (-539.15 kJ/mol). **Conclusion:** The nine active ingredients of *Scurrula atropurpurea* do not interfere with the physiological function of RANKL to interact with RANK. The initial interaction of RANK with catechin or rutin will facilitate the bond of RANK to RANKL. When forming a complex with OPG, epicatechin will facilitate its interaction with RANKL.

**Keywords:** bone loss, diabetes mellitus, docking; herbs, tea parasite.

## 1. INTRODUCTION

Osteoporosis is a systemic skeletal disorder characterized by a decrease in bone strength leading to an increased risk of fracture. Osteoporosis can significantly reduce musculoskeletal function and leads to disability, even mortality (1). Diabetes and osteoporosis are “covert” diseases, making the diagnosis is missed until the complications manifest. Type 2 diabetes mellitus is a risk factor for osteoporosis (2). The relationship between these two diseases is complex and remains controversial (3).

Diabetes decreases osteoclast activity and disrupts osteoblast activity of the bone cells, resulting in low

bone turnover. This will inhibit osteoclasts and osteoblasts by inducing oxidative stress, hyperglycemia, and loss of weight (4, 5). Advanced glycation end-products will interfere with bone formation by suppressing the osteoblastic lineage (6), apoptosis of osteoblast (7), inhibition of osteoblast-specific factors and decreased mineralization *in vitro* (8).

Management of osteoporosis in diabetes mellitus includes lifestyle interventions, optimization of lifestyle control, analysis of the impact of diabetes mellitus treatment on fracture risk, and treatment with anti-osteoporosis (9). Until recently, to the knowledge of the researchers, herbal applications in the treatment of oste-

oporosis related to diabetes mellitus remain rare. An application of hesperidin to mice with type 1 diabetes mellitus can suppress some pro-inflammatory markers and increase serum markers of bone turnover, including osteopontin and osteocalcin, and lower alkaline phosphatase (10). Green tea supplementation has not been able to improve bone mineral content and bone mass in individuals with diabetes mellitus (11). Anhydroicaritin is a prenylated flavonoid found in several species of *Epimedium*. Anhydroicaritin is capable of suppressing osteoclast differentiation and improving bone loss in diabetic rats (12).

The OPG/RANKL/RANK system plays an important role in bone remodeling. RANK is a receptor located on the surface of osteoclasts. RANK ligands are RANKL synthesized and secreted by bone marrow osteoblasts and stromal cells. RANKL activation of RANK will lead to differentiation of osteoclasts and bone resorption. OPG, functioning as the decoy receptor for RANKL, will block this activity (13, 14).

*Scurrula atropurpurea* is a plant that grows on tea trees, known as a parasite species for tea trees. Empirically, these plants have been used by Javanese people to cure cancers (15, 16). *Scurrula atropurpurea* triggers apoptosis in cervical cancer (17). *Scurrula atropurpurea* also acts as an antioxidant. Some of the active components of this plant is the antioxidant quercetin, quercitrin and kaempferol (18-22). Exogenous antioxidant compounds may suppress oxidative stress that can further inhibit inflammation pathways (23). Until recently, *Scurrula atropurpurea* potential for the treatment of osteoporosis related to diabetes mellitus has not been revealed. Specifically, the effects of *Scurrula atropurpurea* on the RANKL/RANK/OPG system has not been revealed, as well.

## 2. AIM

The purpose of the present study was to analyze the *in silico* interaction between the active compounds of *Scurrula atropurpurea* and the RANKL/RANK/OPG system.

## 3. METHODS

### Search for constituent amino acids of RANK, RANKL, and OPG

The constituent amino acid sequences of RANK proteins (GI: 19924309), RANKL (GI: 2612922), and OPG (GI: 2072185) were obtained from the National Center for Biotechnology Information (NCBI) database, the United States National Library of Medicine (NLM), National Institute of Health (NIH) (<http://www.ncbi.nlm.nih.gov>). The 3D structure of RANK, RANKL, and OPG in the \*.sdf file format would be converted into \*.pdb file format using the OpenBabel software (24).

### Search for the structure of the active components of *Scurrula atropurpurea*

The 3D structure of the constituent active compounds of *Scurrula atropurpurea* was obtained from the PubChem Open Chemistry Database. There were nine active compounds: aviculin (CID 10391477), caffeine (CID 2519), catechin (CID: 9064), epicatechin (CID: 72276), kaempferol (CID 5280863), quercetin (CID 5280343), quercitrin (CID 5280459), rutin (CID 5280805), and theobromine (CID

5429). The 3D structure of those various compounds in the \*.sdf file format will be converted into \*.pdb file format using the OpenBabel software (24).

### 3D modeling of protein structure

The 3D structure of the target proteins was predicted by using the SWISS-MODEL web server by means of the homology modeling method. Those 3D protein structures were subsequently validated using the Ramachandran plot analysis (25, 26).

### Protein-ligand docking and visualization

Docking of the active compounds of *Scurrula atropurpurea* with the target proteins was simulated using the HEX 8.0 software (27). The docking protocol consisted of three visualization stages: minimization of rigid-body energy, semi-flexible repairs, and finishing refinements in explicit solvents. Results of the docking were then visualized using the Chimera 1.6.2 and Discovery Studio 4.1 software.

### Analysis of protein-ligand bond interactions

Results of the docking analysis would subsequently be visualized using the Discovery Studio 4.1, LigPlot+ and LigandScout 3.1 software (28, 29). Analysis of protein-ligand bond interactions was performed to determine the number and type of bonds formed, such as hydrogen bonds, hydrophobic bonds, and van der Waals bonds.

## 4. RESULTS

### Interaction of RANKL with the active compounds of *Scurrula atropurpurea*

Table 1 shows the interaction energy between the various active compounds of *Scurrula atropurpurea* with RANKL. Sequentially, those bond energies were RANKL-aviculin (-274.96 kJ/mol), RANKL-rutin (-263.12 kJ/mol), RANKL-quercitrin (-256.98 kJ/mol), RANKL-quercetin (-226.50 kJ/mol), RANKL-kaempferol (-221.65 kJ/mol), RANKL-catechin (-214.85 kJ/mol), RANKL-epicatechin (-211.66 kJ/mol), RANKL-caffeine (-171.73 kJ/mol) and RANKL-theobromine (-161.14 kJ/mol). Molecular docking between nine active compounds of *Scurrula atropurpurea* against the structure of RANKL can be seen in Figure 1.

### Interaction of RANK with the active compounds of *Scurrula atropurpurea*

RANK interaction with the active compounds of *Scurrula atropurpurea* is shown in Table 2. Sequentially, the bond energies were RANK-rutin (-719.26 kJ/mol), RANK-catechin (-680.15 kJ/mol), RANK-caffeine (-654.48 kJ/mol), RANK-theobromine (-651.77 kJ/mol), RANK-quercitrin (-650.68 kJ/mol), RANK-kaempferol (-643.03 kJ/mol), RANK-epicatechin (-641.86 kJ/mol), RANK-quercetin (-641.76 kJ/mol), and RANK-aviculin (-628.62 kJ/mol).

### Interaction OPG with the active compounds of *Scurrula atropurpurea*

Table 3 shows the interaction energy between the active compounds of *Scurrula atropurpurea* and OPG. Sequentially, the bond energies were OPG-epicatechin (-590.09 kJ/mol), OPG-theobromine (-578.08 kJ/mol), OPG-caffeine (-568.88 kJ/mol), RANKL-catechin (-560.63 kJ/mol), OPG-quercitrin (-554.50 kJ/mol), OPG-rutin (-547.91 kJ/mol), OPG-quercetin (-545.75 kJ/mol), OPG-kaempferol

(-544.48 kJ/mol), and OPG-aviculin (-539.15 kJ/mol).

## 5. DISCUSSION

RANKL is expressed in various tissues, including skeletal muscles, thymus, liver, colon, small intestine, adrenal glands, osteoblasts, epithelial cells of the mammary gland, prostate, and pancreas (30). For the first model, we simulated the changes in energy interactions between nine active ingredients with RANKL relative to the interaction between RANK and RANKL. In the present study, energy interaction between the nine active compounds of *Scurrula atropurpurea* and RANKL was greater than that of between RANK and RANKL (-660.95 kJ/mol). This indicates that the bonding of RANK to RANKL is easier than that of the nine active compounds of *Scurrula atropurpurea* to RANKL. In other words, the nine active compounds of *Scurrula atropurpurea* are not easy to form a complex with RANKL. The authors hypothesized that the nine active ingredients of *Scurrula atropurpurea* would not interfere with the physiological function of RANKL. Physiologically, RANKL can activate osteoclasts to regulate the recruitment of progenitor cells for homeostasis and host defense (31).

For the second model, we simulated the energy changes in the interaction of RANK with the nine active ingredients and the bonding to RANKL. Of those nine active compounds, only the RANK-rutin (-719.26 kJ/mol) and RANK-catechin (-680.15 kJ/mol) complexes had less interaction energy than that of RANK-RANKL (-660.95 kJ/mol). Thus, the initial interaction of RANK with catechin or rutin will facilitate the bond of RANK to RANKL. This is in contrast to previous findings that rutin decreases the activity of RANKL (32, 33). In the present study, the initial interaction of RANK with caffeine, theobromine, quercitrin, kaempferol, epicatechin, quercetin, and aviculin, followed by interaction with RANKL, has greater interaction energy than the direct interaction of RANK with RANKL. This indicates that caffeine, theobromine, quercitrin, kaempferol, epicatechin, quercetin and aviculin can act as inhibitors of osteoclast activation. Previous studies have shown that quercetin and kaempferol reduce RANKL-induced osteoclast differentiation (34, 35). Quercitrin suppresses RANKL mRNA expression in osteoblasts and inhibits osteoclast production (36).

In the third model, we simulated the changes in the interaction energy of OPG bond to RANKL relative to OPG bond to the nine active ingredients followed by its interaction with RANKL. The interaction of RANKL with OPG has an interaction energy of -584.74 kJ/mol. Of the nine active ingredients *Scurrula atropurpurea*, only epicatechin would facilitate its interaction with RANKL (-590.09 kJ/mol) when forming a complex with OPG. Meanwhile, eight compounds had energy interactions greater than those of OPG and RANKL. The present study extends pre-

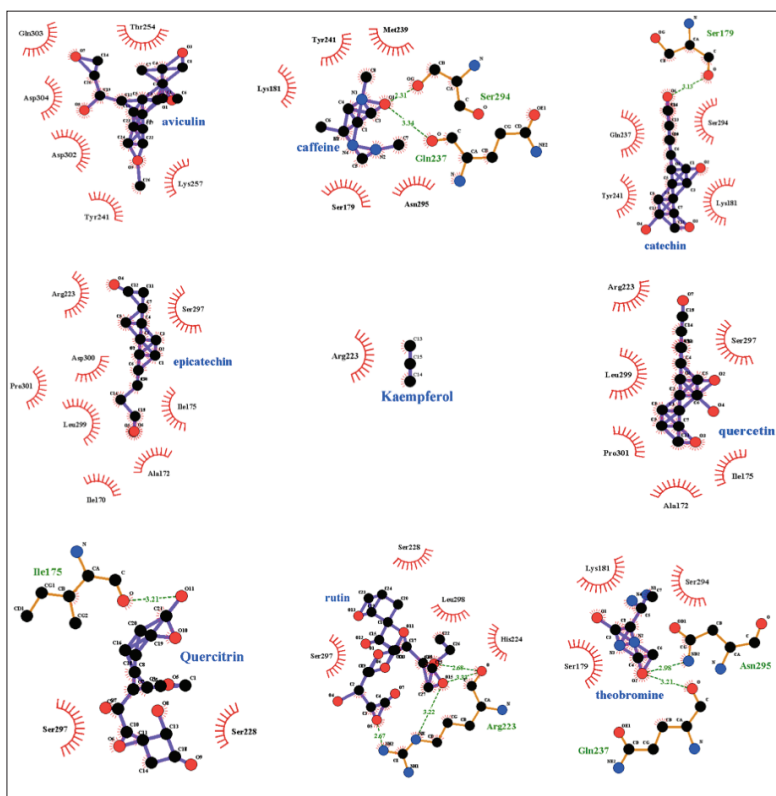


FIGURE 1. The interaction between amino acids in the RANKL structure with nine active ingredients of *Scurrula atropurpurea*.

vious findings that epicatechin can inhibit RANKL-related osteoclastogenesis by suppressing NF- $\kappa$ B signals (37).

## 6. CONCLUSION

The nine active ingredients of *Scurrula atropurpurea* do not interfere with the physiological function of RANKL to interact with RANK. The initial interaction of RANK with catechin or rutin will facilitate the bond of RANK to RANKL. Epicatechin will facilitate its interaction with RANKL when forming a complex with OPG.

- Author's contribution: Study concept and design: I.Z.A.; F.R.P.D.; B.S. Acquisition of data: F.R.P.D. Analysis and interpretation of data: I.Z.A.; F.R.P.D.; B.S. Drafting of the manuscript: I.Z.A.; B.S. Critical revision of the manuscript for intellectual content: I.Z.A.; F.R.P.D.; B.S.
- Conflict of interest statement: There are no conflict of interest
- Financial support and sponsorship: None.

## REFERENCES

1. Edwards BJ. Osteoporosis risk calculators. *Journal of Clinical Densitometry*. 2017; 20(3): 379-388.
2. Romana MS, Li-Yu JT. Investigation of the relationship between type 2 diabetes and osteoporosis using bayesian inference. *Journal of Clinical Densitometry*. 2017; 10(4): 386-390.
3. Garcia-Martin A, Reyes-Garcia R, Garcia-Castro JM, Munoz-Torres M. Diabetes and osteoporosis: Action of gastrointestinal hormones on the bone. *Revista Clinica Espanola*. 2013; 213(6): 293-297.
4. Abbassy MA, Watari I, Soma K. The effect of diabetes mellitus on rat mandibular bone formation and microarchitecture. *Eur J Oral Sci*. 2010; 118: 364-369.
5. Hamada Y, Kitazawa S, Kitazawa R, Fujii H, Kasuga M, Fukagawa M. Histomorphometric analysis of diabetic osteopenia in strepto-

- zotocin- induced diabetic mice: a possible role of oxidative stress. *Bone*. 2007; 40: 1408-1414.
6. Okazaki K, Yamaguchi T, Tanaka K, Notsu M, Ogawa N, Yano S, et al. Advanced glycation end products (AGEs), but not high glucose, inhibit the osteoblastic differentiation of mouse stromal ST2 cells through the suppression of osterix expression, and inhibit cell growth and increasing cell apoptosis. *Calcif Tissue Int*. 2012; 91: 286-296.
  7. Alikhani M, Alikhani Z, Boyd C, MacLellan CM, Raptis M, Liu R, et al. Advanced glycation end products stimulate osteoblast apoptosis via the MAP kinase and cytosolic apoptotic pathways. *Bone*. 2007; 40: 345-353.
  8. Sanguineti R, Storace D, Monacelli F, Federici A, Odetti P. Pentosidine effects on human osteoblasts in vitro. *Ann NY Acad Sci*. 2008; 1126: 166-172.
  9. Chandran M. Clinical aspects and management of osteoporosis and fragility fractures in patients with diabetes. *Osteoporosis & Sarcopenia*. 2017; 3: 123-127.
  10. Shehata AS, Amer MG, El-Haleem MRA, Karam RA. The ability of hesperidin compared to that of insulin for preventing osteoporosis induced by type I diabetes in young male Albino rats: A histological and biochemical study. *Experimental & Toxicologic Pathology*. 2017; 69: 203-212.
  11. de Amorim LMN, Vaz SR, Cesário G, Coelho ASG, Botelho PB. Effect of green tea extract on bone mass and body composition in individuals with diabetes. *Journal of Functional Foods*. 2018; 40: 589-594.
  12. Zheng ZG, Zhang X, Zhou YP, Lu C, Thu PM, Qian C, et al. Anhydroicaritin, a SREBPs inhibitor, inhibits RANKL-induced osteoclastic differentiation and improves diabetic osteoporosis in STZ-induced mice. *European Journal of Pharmacology*. 2017; 809: 156-162.
  13. Kohli SS, Kohli VS. Role of RANKL-RANK/osteoprotegerin molecular complex in bone remodeling and its immunopathologic implications. *Indian J Endocrinol Metab*. 2011; 15 : 175-181.
  14. Liu JZ, Ji ZL, Chen SM. The OPG/RANKL/RANK system and bone resorptive disease, *Sheng Wu Gong Cheng Xue Bao* 2003; 19: 655-660.
  15. Ohashi K, Winarno H, Mukai M, Shibuya H. Preparation and cancer cell invasion inhibitory effects of C16-alkynic fatty acids. *Chem Pharm Bull*. 2003; 51(4): 463-466.
  16. Ohashi K, Winarno H, Mukai M, Inoue M, Prana MS, Simanjuntak P, et al. Indonesian Medicinal Plants. XXV. Cancer cell invasion inhibitory effects of chemical constituents in the parasitic plant *Scurrula atropurpurea* (Loranthaceae). *Chem Pharm Bull*. 2003; 51(3): 343-345.
  17. Parwati NWM, Lindayani IK, Ratnawati R, Winarsih S, Nurseta T. Possible effect of tea plant parasite, *Scurrula atropurpurea* (Blume) Danser, on growth inhibition of culture HeLa cells *in vitro* through DNA repair and apoptosis intrinsic pathways mechanism. *Asian Pacific Journal of Tropical Disease*. 2015; 5(9): 743-746.
  18. Athiroh N, Permatasari N, Sargowo D, Widodo MA. Antioxidative and blood pressure-lowering effects of *Scurrula atropurpurea* on deoxycorticosterone acetate-salt hypertensive rats. *Biomarkers and Genomic Medicine*. 2014; 6(1): 32-36.
  19. Afanasev IB, Dorozhko AI, Brodskii AV, Kostyuk VA, Potapovitch AI. Chelating and free radical scavenging mechanisms of inhibitory action of rutin and quercetin in lipid peroxidation. *Biochem Pharmacol*. 1989; 38: 1763-1769.
  20. Van Acker SA, van Balen GP, van den Berg DJ, Bast A, van der Vijgh WJ. Influence of iron chelation on the antioxidant activity of flavonoids. *Biochem Pharmacol*. 1998; 56: 935-943.
  21. Saija A, Scalese M, Lanza M, Marzullo D, Bonina F, Castelli F. Flavonoids as antioxidant agents: Importance of their interaction with biomembranes. *Free Radic Biol Med*. 1995; 19: 481-486.
  22. Jung HA, Jung MJ, Kim JY, Chung HY, Choi JS. Inhibitory activity of flavonoids from *Prunus davidiana* and other flavonoids on total ROS and hydroxyl radical generation. *Arch Pharm Res*. 2003; 26: 809-815.
  23. Glorie G, Legrand-Poels S, Piette J. NF- $\kappa$ B activation by reactive oxygen species: fifteen years later. *Biochem Pharmacol*. 2006; 72: 1493-1505.
  24. O'Boyle N, Banck M, James CA, Morley C, Vandermeersch T, Hutchison GR. Open Babel: An open chemical toolbox. *J Cheminform*. 2011; 3: 33.
  25. Arnold K, Bordoli L, Kopp J, Schwede T. The SWISS-MODEL workspace: a web-based environment for protein structure homology modelling. *Bioinformatics*. 2006; 22(2): 195-201.
  26. Kiefer F, Arnold K, Kunzli M, Bordoli L, Schwede T. The SWISS-MODEL repository and associated resources. *Nucleic Acids Res*. 2009. 37(Database issue): 387-392.
  27. Macindoe G, Mavridis L, Venkatraman V, Devignes MD, Ritchie DW. HexServer: an FFT-based protein docking server powered by graphics processors. *Nucleic Acids Res*. 2010; 38(Web server issue): 445-449.
  28. Laskowski RA, Swindells MB. LigPlot+: multiple ligand-protein interaction diagrams for drug discovery. *J Chem Inf Model*. 2011; 51(10): 2778-2786.
  29. Wolber G, Langer T. LigandScout: 3-D pharmacophores derived from protein-bound ligands and their use as virtual screening filters. *J Chem Inf Model*. 2005; 45(1): 160-169.
  30. Wada T, Nakashima T, Hiroshi N, Penninger JM. RANKL-RANK signaling in osteoclastogenesis and bone disease. *Trends Molecular Medicine*. 2006; 12(1): 17-25.
  31. Boyce BF, Xing L. Biology of RANK, RANKL, and osteoprotegerin. *Arthritis Research & Therapy*. 2007; 9(Suppl 1): S1.
  32. Rassi CM, Lieberherr M, Chaumaz G, Pointillart A, Cournot G. Modulation of osteoclastogenesis in porcine bone marrow cultures by quercetin and rutin. *Cell Tissue Res*. 2005; 319(3): 383-393.
  33. Kyung TW, Lee JE, Shin HH, Choi HS. Rutin inhibits osteoclast formation by decreasing reactive oxygen species and TNF-alpha by inhibiting activation of NF-kappaB. *Exp Mol Med*. 2008; 40(1): 52-58.
  34. Wattel A, Kamel S, Prouillet C, Petit JP, Lorget F, Offord E, et al. Flavonoid quercetin decreases osteoclastic differentiation induced by RANKL via a mechanism involving NF kappa B and AP-1. *J Cel Biochem*. 2004; 92(2): 285-295.
  35. Chen HJ, Lin CM, Lee CY, Shih NC, Peng SF, Tsuzuki M, et al. Kaempferol suppresses cell metastasis via inhibition of the ERK-p38-JNK and AP-1 signaling pathways in U-2 OS human osteosarcoma cells. *Oncol Rep*. 2013; 30(2): 925-932.
  36. Wu YW, Chen SC, Lai WFT, Chen YC, Tsai YH. Screening of flavonoids for effective osteoclastogenesis suppression. *Anal Biochem*. 2013; 433: 48-55.
  37. Lin TH, Yang RS, Wang KC, Lu DH, Liou HC, Ma Y, et al. Ethanol extracts of fresh *Davallia formosana* (WL1101) inhibit osteoclast differentiation by suppressing RANKL-induced nuclear factor- $\kappa$ B activation. *Evid Based Complement Alternat Med*. 2013; 2013: 647189.