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The interaction of the active compounds of *Labisia pumila* on RANK–RANKL–OPG system

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SUMMARY

The objective of this research was to analyze the interaction of the active ingredients in *Labisia pumila* (kaempferol, rutin, apigenin, caffeic acid, pyrogallol and gallic acid) against RANK–RANKL–OPG system in context of osteoporosis. This is an *in silico* research. The three-dimensional structure in .sdf file is converted to PDB format using the Open Babel 2.3.1 software. The three-dimensional structure model of RCSB RANK–RANKL and RANKL–OPG was obtained from http://www.rcsb.org/pdb. The ligand–protein docking and visualization analysis was carried out with Hex8.0 and Discovery Studio Client 3.5 software. RANK–RANKL or RANKL–OPG complex has the easiest interaction with rutin. In conclusion, flavonoid

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compounds (rutin, apigenin, kaempferol) more easily interact with RANK–RANKL and RANKL–OPG systems than isofavonoid groups (caffeic acid, pyrogallol, gallic acid). This opens the opportunity for the utilization of flavonoid from *L. pumila* as antiosteoporosis.

1. Introduction

The expression of the members of the TNF big family, namely the receptor activator of NF-κB ligand (RANKL) and osteoprotegerin (OPG), which is accompanied by the expression and signaling through the receptor activator of NF-κB (RANK) plays an important role in bone remodeling. RANKL/RANK signaling regulates the formation of osteoclast, activation and survival of normal bone modeling and remodeling, and is involved in various pathological conditions characterized by the increase of bone turnover, such as osteoporosis. OPG protects bones from excessive resorption through binding to RANKL and prevents it to bind to RANK. RANKL and OPG concentrations in the bone will determine bone mass and strength [1]. It is very important to maintain the balance of bone formation and resorption, where the imbalance will cause the decrease in bone mineral density that may trigger the increase in osteoporosis risks [2].

In the last few years, many researchers state that phytochemical materials or plant derivatives components are able to help maintain bone health. *Labisia pumila* or in Malay language known as *Kacip Fatimah* or *Rumput Fatimah* contains high content of phenolic acids and flavonoids. Phenolic acids and flavonoids are believed to play an important role in the plant pharmacological activity. *L. pumila* is widely used as herbal medicines in Southeast Asia for various kinds of diseases [3]. *L. pumila* contains some flavonoids such as apigenin, kaempferol, rutin and myricetin. *Pumila Labisia* contains several phenol such as gallic acid, pyrogallol and caffeic acid [3,4]. Phenolic acids and flavonoids play an essential role in the pharmacological activity in many plants [5].

L. pumila is widely used not only to relieve pain during menstruation, enhance sexual function, but it is also used as an alternative hormone replacement therapy for postmenopausal women [6,7]. Postmenopausal women are susceptible to osteoporosis due to low estrogen. Estrogen plays a role in the form of estrogen receptor-α (ERα) and receptor-β (ERβ) having high affinity against the osteoblasts and osteoclasts [8]. The activation of the estrogen receptor complex is very vital to maintain bone remodeling process [9]. Estrogen can induce and inhibit osteoclast apoptosis, which indirectly will reduce bone resorption and enhance bone formation activity [10]. Thus, the wide range of active compounds of *L. pumila* extract is supposedly used as anti-osteoporosis agent. Data of this research will be analyzed *in-silico* basis to determine the interaction of the active ingredients in *L. pumila* (kaempferol, rutin, apigenin, caffeic acid, pyrogallol and gallic acid) against RANK–RANKL–OPG system.

2. Material and methods

2.1. Ligand preparation

The 3D-structures of kaempferol, rutin, apigenin, caffeic acid, pyrogallol and gallic acid compounds were obtained from NCBI PubChem (kaempferol ID: CID5280805, rutin ID: CID5280805, apigenin ID: CID5280443, pyrogallol ID: CID1057 and gallic acid ID: CID370). After that, the 3D structures are in the form of file.sdf converted into PDB format using Open Babel 2.3.1 software.

2.2. Protein receptor of RANK–RANKL–OPG

The 3D-structure model of RANK–RANKL PDB ID: 3ME2 and RANKL-OPG PDB ID 4E4D was obtained from http://www.rcsb.org/pdb. The protein validation was analyzed by Ramachandran plot [11–13].

2.3. Ligand–protein docking and visualization

Software Hex8.0 is a rigid docking used as a device to calculate the interaction probability of kaempferol, rutin, apigenin, caffeic acid, pyrogallol and gallic acid with RANK–RANKL and RANKL–OPG. The result will illustrate the interaction of their active properties and their visualization and Ramachandran plot is analyzed using Discovery Studio Client 3.5 software [14,15].

3. Results

The results of docking between RANK–RANKL and RANK–OPG complex bonds with the active ingredients of *L. pumila* (kaempferol, rutin, apigenin, caffeic acid, pyrogallol and gallic acid) show that all compounds from *L. pumila* interact with RANK–RANKL and RANK–OPG complex. RANK–RANKL complex has the easiest interaction with rutin (−313.0 kJ/mol) and has the most difficult interactions with pyrogallol (−136.3 kJ/mol) (Table 1).

Meanwhile, the complex interaction of RANKL–OPG with the active ingredients, in which the RANKL–OPG complex has the highest interaction with rutin (−322.0 kJ/mol) and has the lowest interaction with pyrogallol (−136.3 kJ/mol) (Table 2).

4. Discussion

Bone remodeling involves the balance between bone formation and resorption. The disruption of the balance between the two processes will trigger bone pathology. There are several factors or cytokines known to play important roles in bone remodeling process. The bone remodeling is regulated by receptor activator of nuclear factor kappa-B ligand (RANKL) and osteoprotegerin (OPG), produced by osteoblasts [16] RANK binds to RANKL receptors located in osteoclast precursors to trigger differentiation into mature osteoclasts and then provokes bone resorption [17]. The study indicates that the administration of RANKL serum to mice may trigger the growth and activation of osteoclasts that leads to osteoporosis [18]. The docking results show the interaction between the active ingredients of *L. pumila* with RANK–RANKL complex. The RANK–RANKL complex has the highest interaction with rutin (−313.0 kJ/mol) and has the lowest interaction with pyrogallol (−136.3 kJ/mol). It indicates that the rutin is the potential active ingredient that is able to interact with RANK–RANKL complex the most. Previous research has shown that rutin can inhibit osteoclast formation induced by RANKL [19].

Meanwhile, as for the interaction in the system of RANKL–OPG, OPG acts as the anti-resorption receptor that binds RANKL and prevents it from binding to RANK. As a result, OPG inhibits osteoclast differentiation and bone resorption activity. In this research, there is the interaction between RANKL–OPG complex and the active ingredient, in which the RANKL–OPG complex has the highest interaction with rutin (−322.0 kJ/mol) and has the lowest interaction with pyrogallol (−136.3 kJ/mol). It also indicates that rutin is the most potential active ingredient that is easiest to interact with RANKL–OPG complex. Our finding extends previous study that rutin can inhibit osteoclast formation induced by RANKL [19]. The rutin interaction with RANKL–RANK and RANKL–OPG systems confirms its role as an antiosteoporosis agent, consistent with previous studies [20].

Table 1
Total energy of the interaction between RANK–RANKL and active ingredients of *Labisia pumila*.

No	Interaction	Total energy (kJ/mol)
1	RANK-RANKL	−705.6 kJ/mol
2	RANK-RANKL + rutin	−313.0 kJ/mol
3	RANK-RANKL + apigenin	−267.8 kJ/mol
4	RANK-RANKL + kaempferol	−258.0 kJ/mol
5	RANK–RANKL + caffeic acid	−197.6 kJ/mol
6	RANK–RANKL + gallic acid	−177.9 kJ/mol
7	RANK-RANKL + pyrogallol	−136.3 kJ/mol

Table 2
Total energy of the interaction between RANKL–OPG and active ingredients of *Labisia pumila*.

No	Interaction	Total energy (kJ/mol)
1	RANKL-OPG	−814.1 kJ/mol
2	RANKL-OPG + rutin	−322.0 kJ/mol
3	RANKL-OPG + kaempferol	−229.8 kJ/mol
4	RANKL-OPG + apigenin	−229.1 kJ/mol
5	RANKL–OPG + caffeic acid	−178.2 kJ/mol
6	RANKL–OPG + gallic acid	−161.3 kJ/mol
7	RANKL-OPG + pyrogallol	−136.3 kJ/mol

Isoflavonoid components include pyrogallol, caffeic acid, and gallic acid. Meanwhile, kaempferol, rutin and apigenin, are classified as a flavonoid component. Thus, in this study, it has been proven that the interaction of RANK–RANKL and RANKL–OPG is easier on the flavonoid compound than isoflavonoid compound of *L. pumila*. This leads to the ease of interaction of flavonoids versus isoflavonoid will increase the effectiveness in bone and reduce side effects. These findings expanded earlier findings that isoflavonoid may provide side effects in during long-term administration of estrogen sensitive organs [21].

In conclusion, flavonoid compounds (rutin, apigenin, kaempferol) more easily interact with RANK–RANKL and RANKL–OPG systems than isofavonoid groups (caffeic acid, pyrogallol, gallic acid). This opens the opportunity for the utilization of flavonoid from *L. pumila* as antiosteoporosis.

Conflict of interest

None.

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