EasyChair Preprint

№ 7186

In-Silico Test of Activity Genistein as a Natural Compound Against Osteoporosis in Post Menopausal Women

Trinarti Senolangi, Ika Yustisia, Andi Ariyandy and Sulfahri

December 8, 2021

IN-SILICO TEST OF ACTIVITY GENISTEIN AS A NATURAL COMPOUND AGAINST OSTEOPOROSIS IN POST MENOPAUSAL WOMEN

Senolangi T[1)], Yustisia I[1)], Ariyandi A[2)], Sulfahri[3)]

[1)]Biomedical Sciences, Postgraduate School, Hasanuddin University, Makassar, Indonesia
[2)]Department of Physiology, Faculty of Medicine, Hasanuddin University, Makassar, Indonesia
[3)]Department of Biology, Faculty of Mathematics and Natural Sciences, Hasanuddin University, Makassar, Indonesia

E-mail: trinarti76@gmail.com

Abstract

Sauropus androgynus is widely used in traditional medicine for wound healing, inducing lactation, relief of urinary disorders, as an antidiabetic cure and also fever reduction. This study aimed to discover bioactivity of the genistein compound from *Sauropus androgynus* for the treatment of osteoporosis, especially in postmenopausal women. Structures of chemical constituents of *Sauropus androgynus* (genistein) was collected from the literature. The water molecule and ligands were removed by using PyMOL Software v1.7.4.5 (Schrodinger). Docking of target proteins were performed using the PyRx 0.8 software. Prediction and significant descriptors of Physicochemical properties, Lipophilicity, Pharmacokinetics and Druglikeness properties of the compounds were predicted using Swissadme. The results showed that genestein compounds has greater potential as an antiosteoporosis compared to the control compounds, based on its binding affinity and intermolecular interactions, the binding affinity of genistein with estrogen receptor alpha (ERα) protein is -7.3, while binding affinity estrogen receptor alpha (ERα) protein with the control compound alendronate is -4.5. AMES test showed that genistein is not a potential mutagens and not carcinogens. Druglikeness prediction showed that genestein fulfil the rules of Lipinski, Ghose, Veber, Egan and Muegge with 0.55 Bioavailability Score.

Keywords: antiosteoporosis, *Sauropus androgynus*, genistein, alendronate, estrogen receptor alpha (ERα)

1. Introduction

Osteoporosis is a metabolic disease of the bones characterized by reduced bone minerals and bone matrix resulting in decreased bone strength[4]. In menopause, estrogen deficiency impairs the normal cycle by increasing osteoclastic resorption activity without a corresponding increase in osteoblastic activity and therefore the amount of bone resorbed is greater than the amount deposited leading to bone loss[5]. Osteoporosis becomes a serious public health problem, which can lead to pain, disability, loss of functional independence and increased morbidity and mortality. Women have more osteoporosis than men, with the number of cases increasing after menopause[3]. The incidence of osteoporosis-related fractures is expected to increase substantially over the coming decades. It is estimated that the number of annual fractures in the European Union will increase from 3.5 million in 2010 to 4.5 million by 2025 [11].

Traditional medicine derived from plants as an alternative medicine is recommended by the World Health Organization (WHO), as it is considered effective, with minimal side effects and relatively low cost. Sauropus androgynus L. Merr. It is a plant that belongs to

the family Euphorbiaceae. Sauropus androgynus is one of the most popular herbs in South Asia, Southeast Asia, and China. Some studies explain that excessive consumption of Sauropus androgynus can cause drowsiness, constipation, and bronchiolitis obliterans and can lead to respiratory failure. Interestingly, this herb has been used in Malaysia and Indonesia in cooking and is commonly called a "multigreen" or "multivitamin" plant due to its high nutritional value and cheap food protein source[1].

Sauropus androgynus is widely used in traditional medicine for wound healing, lactation, relieving urinary disorders, as an antidiabetic drug and also a fever-lowering. Research into the phytochemicals of Sauropus androgynus leaves revealed they contain sterols, resins, tannins, saponins, alkaloids, flavonoids, terpenoids, glycosides, phenols, catechols, cardiac glycosides, and acidic compounds. In this study, we found the bioactivity of Sauropus Androgynus contains flavonoids (genisein) for antiosteoporosis based on Reverse Docking and ADME predictions.

2. Materials and Method

2.1. Ligands Preparation

Structures of the chemical compound of genistein was collected from published literature. Chemical 3D structure and SMILES of ligand *Sauropus androgynus* (genistein) taken from PubChem compound database (https://pubchem.ncbi.nlm.nih.gov/) with number ID: CID 5280961 and Canonical Smile : C1=CC(=CC=C1C2=CO C3=CC(=CC(=C3C2=O)O)O)O. The two dimensional (2D) and the three-dimensional (3D) chemical structures of the ligands were sketched using Avogadro and were saved in PDB format.

2.2. Target Selection

The molecular structure taken from literature and validate using Uniport (https://www.uniprot.org). The protein that was collected and validated with PDB (Protein Data Bank https:///www.rcsb.org/pdb). The water molecule and ligands were removed by using PyMOL v1.7.4.5 Software (Schrödinger). In this study, the target protein used was estrogen receptor alpha (REα) with the 2OUZ code of PDB. Estrogen receptor (ER)-α is the main mediator of these estrogenic effects in bone. Therefore, estrogen signaling via ERα is a target both for affecting longitudinal bone growth and for bone remodeling[2]

2.3. Molecular Docking

Molecular docking experiments were performed using the PyRx 0.8 software. The reverse docking process was carried out using the Vina Wizard feature integrated into PyRx 0.8 software which reacts to the natural compound genistein, the target protein estrogen receptor alpha (REα) and the control compound (activator compound estrogen receptor alpha (REα)). Activator compounds will be a positive control in the docking process. The activator compound of estrogen receptor alpha (REα) is alendronate. In women with documented osteopenia, or a strong family history of osteoporosis, a low dose of alendronate has been shown to increase and maintain bone density and reduce fractures[8].

2.4. Visualization of Molecule and Small Molecule Interaction

The interactions between ligands (genistein), target protein (estrogen rceptor alpha (ERα) and known inhibitors of target protein (alendronate) visualized and analyzed using PyMol v1.7.4.5 Software.

2.5. Compound's Properties and ADMET Predictions

Swissadme (http://www.swissadme.ch) and admetSAR (lmmd.ecust.edu.cn) is used to predict the prediction and significant descriptors of Physicochemical Properties, Lipophilicity, Pharmacokinetics and Druglikeness properties of the compounds.

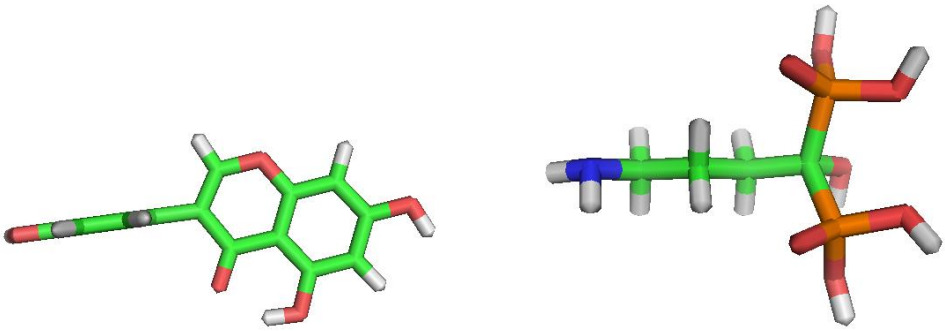
3. Results and Disscussion

Based on the literature study, it was found that sauropus androgynus has a relationship with the alpha estrogen receptor. The main compounds found in Sauropus androgynus is genistein. genistein compounds are known to interact with one type of protein in osteoblast and osteoclast cells[9]. Genistein is a phytoestrogen, a chemical similar to estrogen in plants that functions as a precursor in human metabolism. Phytoestrogens naturally become chemicals that can interact with estrogen receptors to weaken estrogen or in other words as antiestrogens. Genestein has the best potential as an estrogen replacement hormone because it has the highest energy affinity among other isoflavones for ERα. Genistein is able to modulate ERα expression, and thus potentially alter osteocyte mechanosensitivity[7]. A clinical trial also demonstrated its beneficial effects on improvement of bone mass in postmenopausal women.21 As to the mechanisms, genistein inhibits osteoclast activity viaactivating nuclear factor-kappaB (NF-κB) and stimulates maturation of osteoprogenitors and osteoblasts through ERα/β-dependent initiation of the p38mitogen-activated protein kinase (MAPK)-Runx2 mechanism[12].

The process of binding the hormone estrogen to estrogen receptors in the cell membrane, and binds in the form of dimers. Once the hormone binds to its receptor, the receptor moves to the cell nucleus and then binds to ERE (estrogen response element). Furthermore, the complex will bind to the activator so that transcription factors become active that can change gene expression. Then the regulation of gene transcription will produce a specific protein that is involved in certain biological functions[6]. Bisphosphonates (alendronate) suppress bone turnover, prevent bone loss and preserve bone architecture by tightly adhering to bone surfaces and by inhibiting the enzyme farnesyl pyrophosphate synthase which is required for formation of the cytoskeleton in osteoclasts, thereby inhibiting bone resorption[8].

Structure of genistein with alendronate and estrogen receptor alpha, visualized in 3 dimensions (3D) using PyMol. Through reverse docking technique can be known the potential of genistein has the potential as antiosteoporosis in postmenopausal women. Based on reverse docking results, the binding affinity of estrogen receptor alpha protein to alendronate showed lower binding affinity than estrogen receptor alpha protein to genistein.

The number of binding affinities illustrate the potential of a compound or a ligand to interact with protein target. If the ligand has lower binding affinity, it will be stronger to inherit the protein target. Hence, the lower binding affinity leads the lower energy needed of ligand to interact with the protein target[10]. The estrogen receptor alpha has an interaction with natural compounds from the sauropus androgynus plant that has been visualized using PyMol software. The binding affinity of genistein with estrogen receptor alpha is -7.3, while the binding affinity of alendronate with estrogen receptor alpha is -4.5. Based on the results of the study, comparing the strengthen of genistein with alendronate to estrogen receptor alpha has shown that genistein has an ability to bind to estrogen receptor alpha.



(a) **(b)**

Figure 1. (a) Chemical 3D Structure of Genistein and (b) Alendronate were showed by software PyMol

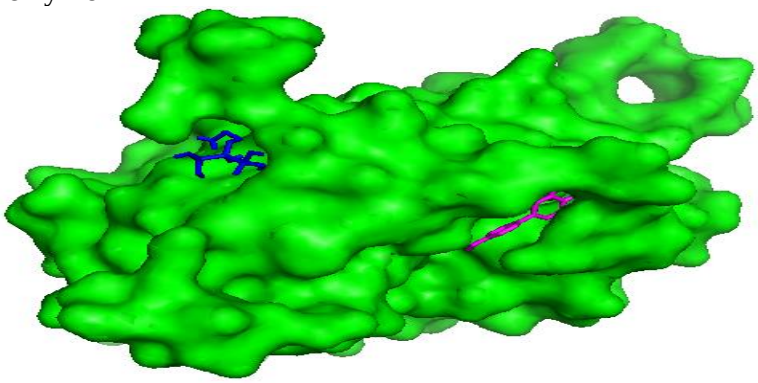


Figure 2. Binding Site of genistein (purple), alendronate (blue) with receptor alpha ERα.

Table 1. The result of Reverse Docking estrogen receptor alpha (ERα) with ligand and control activator

Ligand	**Binding Affinity**
ERα and genistein	-7,3
ERα and alendronate	-4,5

In this study, ADMET predictions were made to determine the side effects of genistein compounds for the body that were evaluated and associated with cell permeation, metabolism process and bioavailability. AMES test results show that genistein is not potential mutagens and not carcinogens. The Ligands is considered to have the potential to enter the cell membrane and be absorbed by the body if they meet Lipinski's rules. The search results show that α-caesalpin fulfils the rules of Lipinski, Ghose, Veber, Egan and Muegge with the Bioavailability Score 0.55.

4. Conclussion

This study proved that genistein has potential as an antiosteoporosis in menopausal women based on its binding affinity with -7.3 and intermolecular interactions. *Sauropus androgynus* contains genistein which is potential antiosteoporosis in menopausal women. drug according to Lipinski, Ghose, Veber, Egan dan Muegge rule and 0.55 Bioavailability Score.

Refferences

[1] Bunawan, Hamidun, Siti Noraini Bunawan, Syarul Nataqain Baharum, and Normah Mohd Noor. "Sauropus Androgynus (L.) Merr. Induced Bronchiolitis Obliterans: From Botanical Studies to Toxicology." *Evidence-based Complementary and Alternative Medicine* 2015 (2015).

[2] C.Ohlsson, A. E. Börjesson · M. K.Lagerquist S. H. Windahl ·. "The Role of Estrogen Receptor α in the Regulation of Bone and Growth Plate Cartilage." *Cellular and Molecular Life Sciences* (2013).

[3] Daly, Robin M., Jack Dalla Via, Rachel L. Duckham, Steve F. Fraser, and Eva Wulff Helge. "Exercise for the Prevention of Osteoporosis in Postmenopausal Women: An Evidence-Based Guide to the Optimal Prescription." *Brazilian Journal of Physical Therapy* 23, no. 2 (2019): 170–180. https://doi.org/10.1016/j.bjpt.2018.11.011.

[4] Hingorjo, Mozaffer Rahim, Sadiqa Syed, and Masood A. Qureshi. "Role of Exercise in Osteoporosis Prevention - Current Concepts." *Journal of the Pakistan Medical Association* 58, no. 2 (2008): 78–81.

[5] J Christopher Gallagher, MD* and Sri Harsha Tella, MD. "Prevention and Treatment of Postmenopausal Osteoporosis." *J Steroid Biochem Mol Biol* (2015): 155–170.

[6] Mulyati, Budi. "Peran Genestein Pada Pengikatan Reseptor Estrogen α." *Indept* 8, no. 2 (2019): 1–9. http://jurnal.unnur.ac.id/index.php/indept/article/viewFile/284/272.

[7] Mulyati, Budi. "Tempe Sebagai Pengganti Hormon Estrogen Pada Reseptor Estrogen α Dengan Metode Autodock Vina." *CHEESA: Chemical Engineering Research Articles* 1, no. 1 (2018): 7.

[8] Pérez-lópez, Faustino R. "Postmenopausal Osteoporosis and Alendronate." *THE EUROPEAN MENOPAUSE JOURNAL* 48 (2004): 179–192.

[9] Sihombing, Iknes, Sunny Wangko, and Sonny J.R. Kalangi. "Peran Estrogen Pada Remodeling Tulang." *Jurnal Biomedik (Jbm)* 4, no. 3 (2013).

[10] Sulfahri, A. R. Arif, I. W. Iskandar, and R. Wardhani. "In Silico Approach of Antidiabetic Compounds from Caesalpinia Crista Seed through Docking Analysis and ADMET Predictions." *Journal of Physics: Conference Series* 1341, no. 2 (2019).

[11] Willems, Hubertine M.E., Ellen G.H.M. van den Heuvel, Ruud J.W. Schoemaker, Jenneke Klein-Nulend, and Astrid D. Bakker. "Diet and Exercise: A Match Made in Bone." *Current Osteoporosis Reports* 15, no. 6 (2017): 555–563.

[12] Wu, Gong Jhe, Jui Tai Chen, Yih Giun Cherng, Chuen Chau Chang, Shing Hwa Liu, and Ruei Ming Chen. "Genistein Improves Bone Healing via Triggering Estrogen Receptor Alpha-Mediated Expressions of Osteogenesis-Associated Genes and Consequent Maturation of Osteoblasts." *Journal of Agricultural and Food Chemistry* 68, no. 39 (2020): 10639–10650.