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POSTMENOPAUSAL OSTEOPOROSIS: CHRYSIN EXHBITS ESTROGEN-LIKE ACTIVITY IN SILICO

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ABSTRACT

Postmenopausal osteoporosis is a metabolic bone disease characterized by loss of bone tissue, deterioration of bone microstructure and increase skeletal fragility fracture in the wrist, spine and hip. Estrogen has been implicated in bone formation via estrogen receptor–dependent mechanism. Estrogen receptors (ERs) are members of the nuclear receptor family which are involved in the regulation of several physiological processes including cell growth, survival and differentiation. Molecular docking is a technique use for investigatigating the association of ligand to a target protein and also to elucidate the binding properties of ERs. Several natural compounds derived from food or plant sources have demonstrated the ability to binds to estrogen receptors and have shown interesting estrogenic activity. Chrysin and 17β-estradiol were docked into the binding pocket of both alpha and beta estrogen receptors. The result demonstrated that chrysin and 17β-estradiol display similar interaction pocket with both hydrogen and hydrophobic interface. The energy of the interaction and binding affinity for chrysin was almost similar with that of 17β-estradiol. Thus, the present data reported that chrysin molecule can effectively binds to both ERs in almost same binding site with that of 17β-estradiol and enhances the sensitivity and function of estrogen receptors**.** Altogether, chrysin molecule could be of particular interest and could be useful ingredient in the formulation of nutraceuticals against postmenopausal osteoporosis.

Keywords: Postmenopausal osteoporosis; chrysin; 17β-estradiol; Molecular docking; Estrogen receptors; ligand interactions

INTRODUCTION

Bone is dynamic and metabolically active tissue which is continuously remodeled, shaped and repaired through its lifetime by the coordinated action of osteoclasts and osteoblasts (Mada *et al.,* 2017a; Zhang *et al.,* 2013; Li *et al.,* 2014). Bone metabolism is characterized by an intimate function of osteoblast cells are derived from mesenchymal stem cells which is responsible for bone forming whereas osteoclast cells derived from hematopoietic cells participate in bone resorption (Reddi *et al.,* 2016; Mada *et al.,* 2017b). Imbalance between bone resorption and bone formation in favor of bone resorption can lead to postmenopausal osteoporosis (Mada *et al.,* 2020; Huang *et al.,* 2015). Postmenopausal osteoporosis is a metabolic bone disease characterized by the loss of bone tissue, deterioration of bone microstructure and increase skeletal fragility fracture in the wrist, spine and hip (Reddi *et al.,* 2019; O'Brien *et al.,* 2014; Kanis *et al.,* 2009). The decline in estrogen level has been linked to bone loss during the onset of menopause (Khedgikar *et al.,* 2015; Farr *et al.,* 2013). During menopause condition, the osteoprotective effect of estrogen is drastically reduced leading to increase expression of bone-resorbing cytokines which promote osteoclastogenesis and osteoclastic activity (Mada *et al.,* 2018; Nicole *et al.,* 2015; Yasuda *et al.,* 2013; Almeida *et al.,* 2010; Nakamura *et al.,* 2007). Estrogen receptors (ERs) are members of the nuclear receptor family involved in the regulation of several physiological processes such as cell growth, survival and differentiation (Sukocheva, 2018; Guillaume *et al.,* 2017; Wang *et al.,* 2013). ERs are the natural target of estrogen specifically 17β-estradiol (Fig. 1a). Molecular docking is a technique use for investigatigating the association of ligand to a target protein and also to elucidate the binding properties of ERs (Martinez-Archundia *et al.,* 2018; Yugandhar *et al.,* 2017; Muchtaridi *et al.,* 2017).

Several bioactive compounds derived mainly from food or plant sources have demonstrated ability to binds to estrogen receptors and have shown interesting estrogenic activity (Ibrahim *et al.,* 2021). Chrysin (5, 7-dihydroxyflavone) is a natural flavonoid (Fig. 1b) present in honey, propolis and many plant extracts, and is a major component of traditional medicinal herbs, (Phan *et al.,* 2011; Pushpavalli *et al.,* 2010). Previous study described that chrysin possesses anti-inflammatory and antioxidative effect and exert beneficial effect in the regulation of reproductive system and hormones (Sobocanec *et al.,* 2006). Thus, natural or synthetic compounds capable of binding and enhancing estrogen receptor sensitivity and functions could be useful ingredient in the formulation of nutraceutical agent against postmenopausal osteoporosis. Hence, the present study investigate the potential beneficial effect of chrysin against postmenopausal osteoporosis in silico by targeting ERs.

MATERIALS AND METHODS

The crystal structure of alpha and beta estrogen receptors were downloaded from the protein data bank (www.rcsb.org/pdb) with PDB ID; 5UFW and 2NV7, respectively. The 3D structure of chrysin was obtained from PubChem database (PubChem ID; 5281607). While, the structure of estrogen (17β-estradiol) was drawn using ChemDraw Ultra v12.0.2 and the file converted to pdb format. The structures were prepared by removing all solvent molecules, co-crystallized ligands and optimized to simulate physiological conditions using Chimera v 1.1. Polar hydrogens were added and partial charges were assigned to the standard residue using Gasteiger partial charge. Gasteiger

partial charge algorithm assumes that all hydrogen atoms are represented explicitly. The most favorable binding interactions were determined by molecular docking studies using the PatchDock server (www.bioinfo3d.cs.tau.il/PatchDock) as described previously (Kaushik *et al.*, 2013; Nisha *et al.*, 2016). The interaction was set as protein-small ligand and 4.0 was set as the clustering root mean square deviation (RMSD) of atomic position. This RMSD value help in selecting the most stable conformations by comparison between different ligand pose. The interaction of the docked complex was studied visually with the help of Discovery Studio 2017 R2 Client (v17.2.0.16349). In addition to visualization, the software can be used in structure and macromolecule design, antibody modeling, predictive QSAR, ADMET, X-ray and pharmacophore and ligand based design. Estrogen (17β-estradiol) was used in this study as a reference endogenous estrogen in order to compare docking energies with that of chrysin (Babangida *et al.*, 2018).

RESULT

Comparative effect of chrysin and 17β-estradiol on ERs in silico

The structural chemistry of chrysin and 17β-estradiol shown the presence of two hydroxyl groups in aromatic ring in both the compounds which contribute to the observed polar and nonpolar interactions (Figure 1). Also the presence of carbonyl carbon and a methyl group in chrysin and 17β-estradiol respectively, can enhance and strengthen the interactions. Moreover, about 600 ligands poses within the binding pocket of estrogen receptor were generated for both chrysin and 17β-estradiol. The best docked conformations of the two compounds within the binding site of estrogen receptors were shown (Figures 2 & 3). Systemic molecular docking analysis indicated that chrysin exhibits affinity to 17β-estradiol binding locus on both alpha and beta estrogen receptors (Figures 4 & 5). In addition, other non-conventional interactions involving aromatic pi bond, hydrophobic side chain and unfavorable clashes were illustrated (Figures 6 & 7).

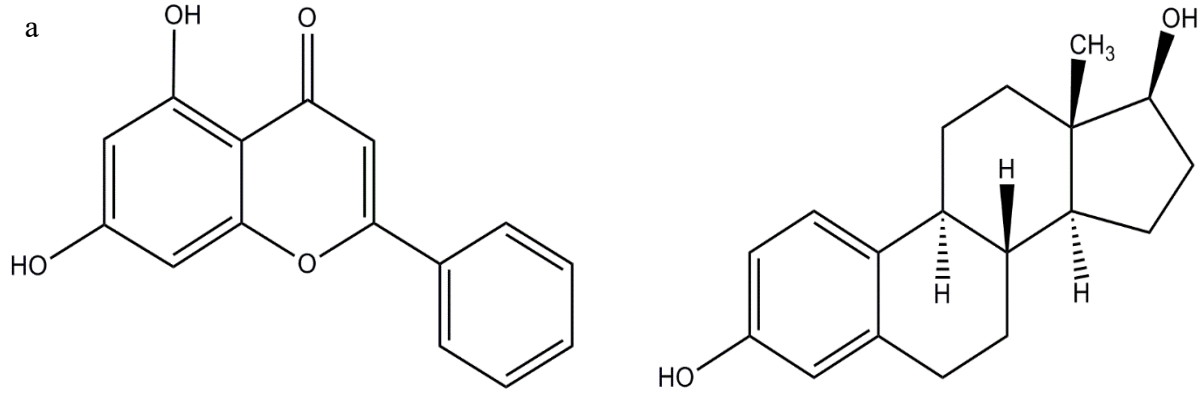

Figure 1: Structure of a) Chrysin downloaded from PubChem database and structure of b) 17β-estradiol drawn using ChemDraw Ultra v12.0.2.

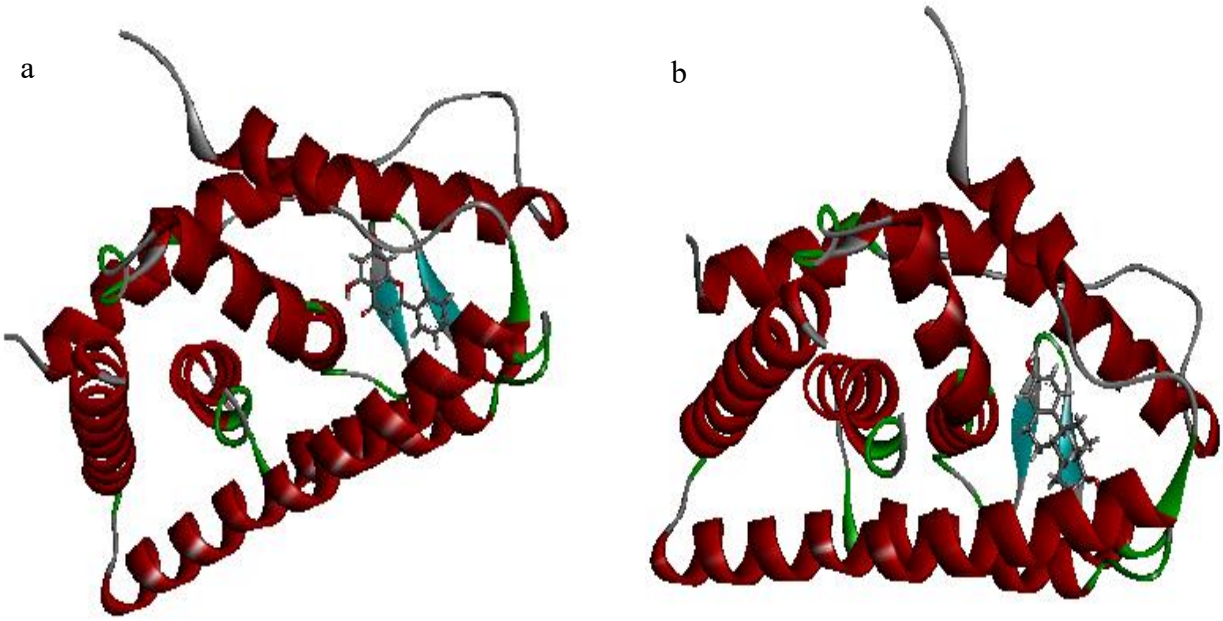

Figure 2**:** Molecular docking interactions at the binding site of alpha estrogen receptor with a) chrysin and b) 17β-estradiol.

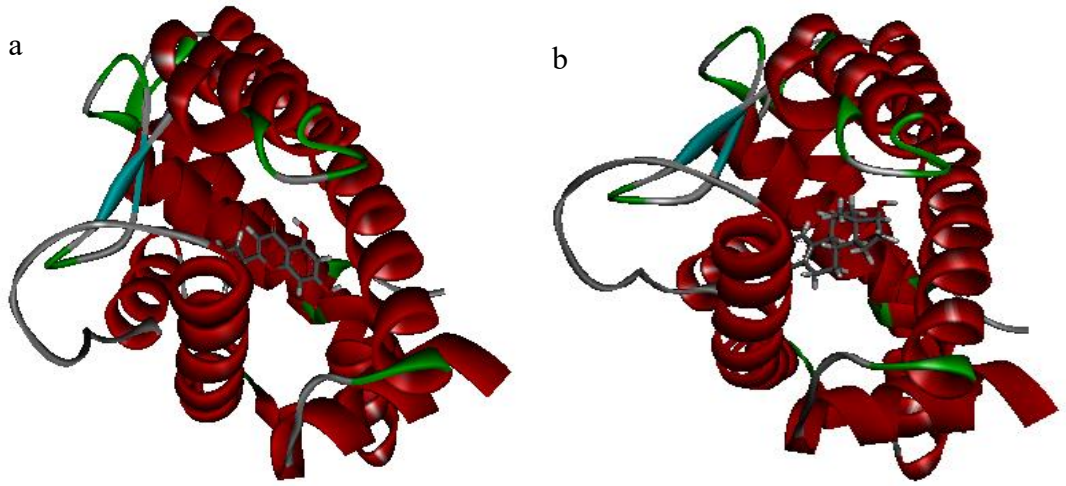

Figure 3**:** Molecular docking interactions at the binding site of beta estrogen receptor with a) Chrysin and b) 17β-estradiol.

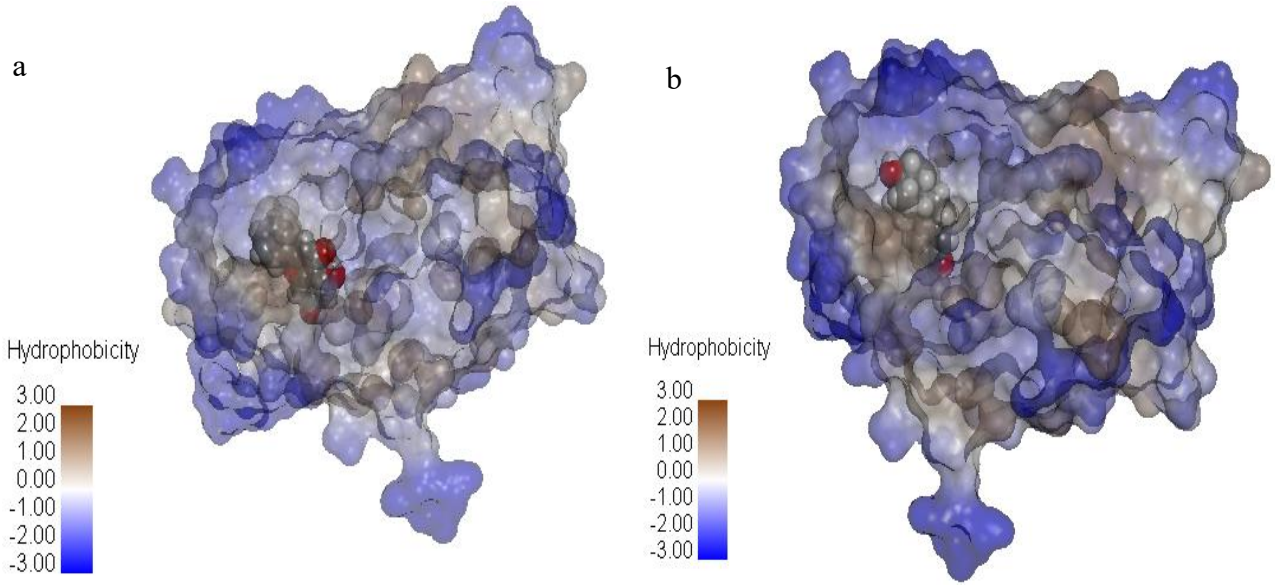

Figure 4**:** Hydrophobic surface interaction in the binding site of alpha estrogen receptor, a) Chrysin and b) 17β-estradiol.

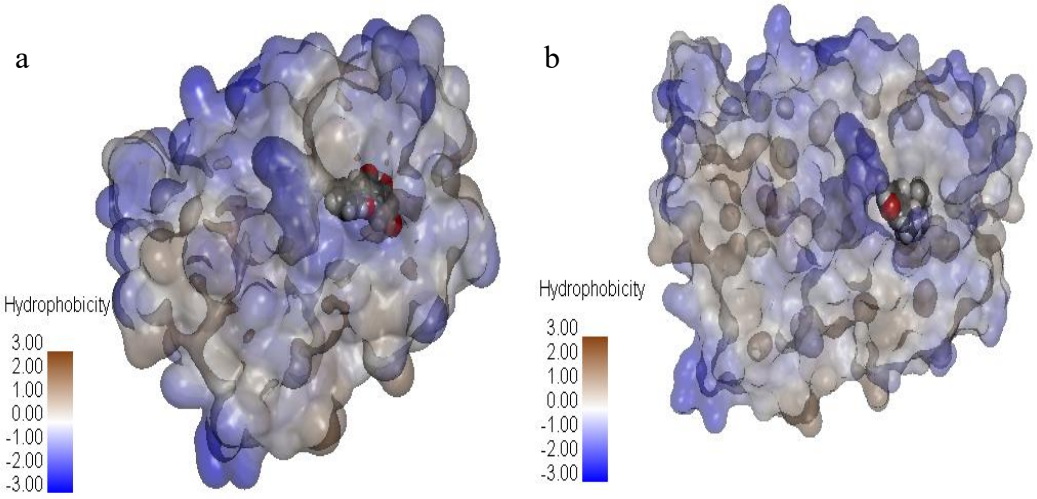

Figure 5**:** Hydrophobic surface interaction in the binding site of beta estrogen receptor, a) Chrysin and b) 17β-estradiol.

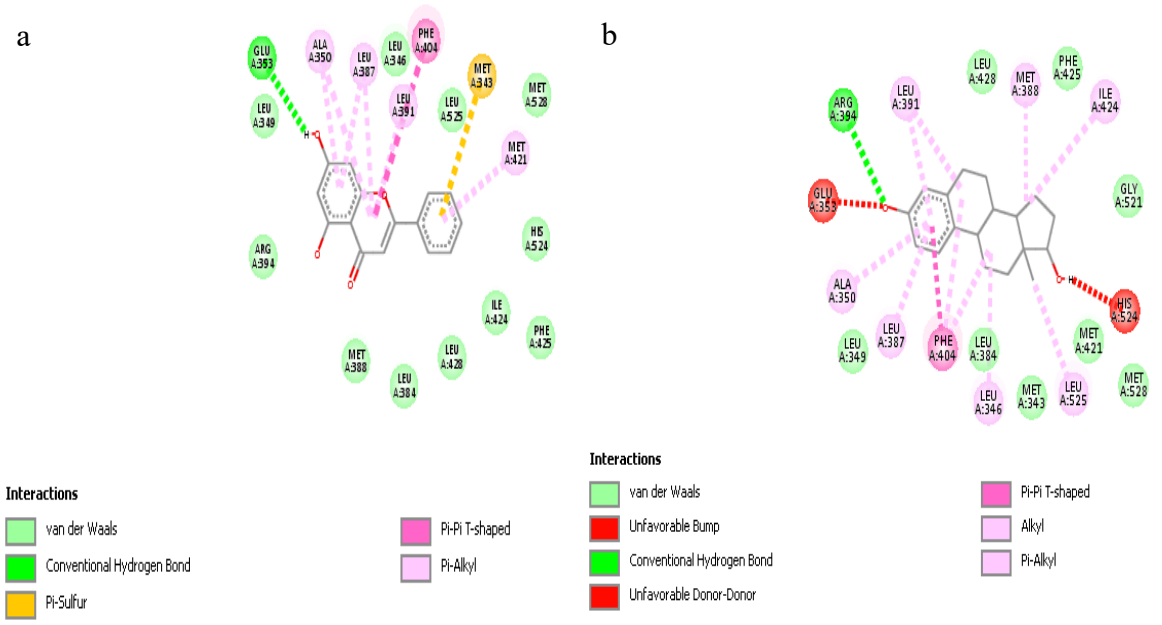

Figure 6: Bonding interactions and amino acids around the binding site of alpha estrogen receptor with a) Chrysin and b) 17β-estradiol.

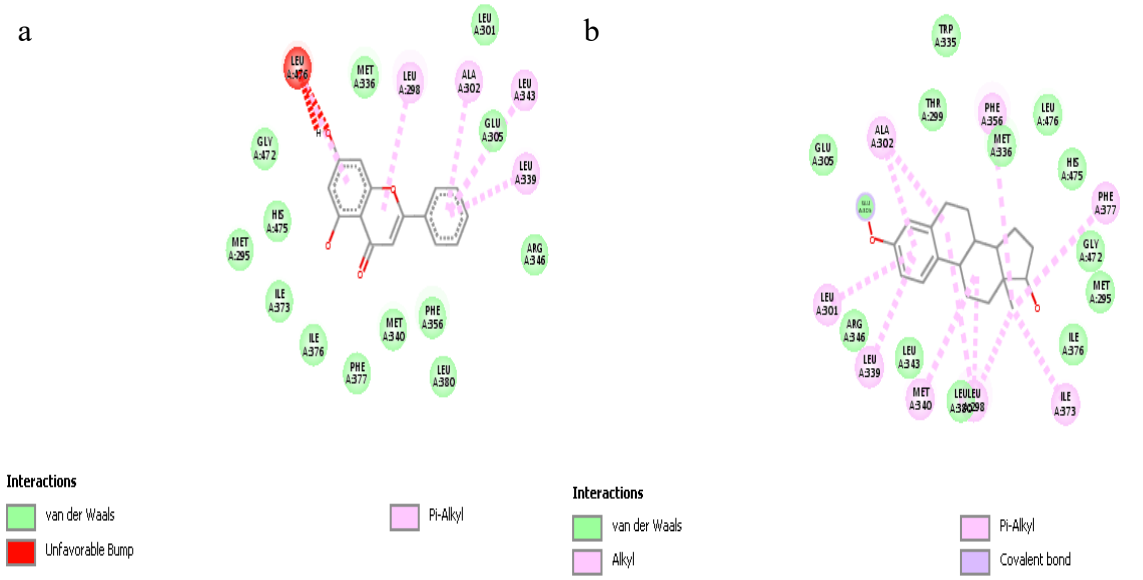

Figure 7**:** Bonding interactions and amino acids around the binding site of beta estrogen receptor with a) Chrysin and b) 17β-estradiol.

Table 1: Binding energy and inhibition binding constant of chrysin and 17β-estradiol

	Chrysin		17β-estradiol	
	Binding Energy (kcal/mol)	**Inhibition Constant(μM)**	**Binding Energy (kcal/mol)**	**Inhibition Constant (μM)**
Alpha Estrogen Receptor	-229.83	0.679	-261.07	0.612
Beta Estrogen Receptor	-252.72	0.653	-290.78	0.644

Furthermore, in alpha receptor, amino acids such as Glu353 was involved in hydrogen bond with 5-OH of chrysin, whereas Leu346, 525, 428, 384, 349 formed Vander Waals, Ala350, Leu387, 391, Met421 were involved in pi-alkyl with aromatic π bond of chrysin and their hydrophobic side chain, Met343 was linked to pi-sulfur with aromatic π bond of chrysin and sulfur side chain of methionine and Phe404 formed π -π interaction with aromatic side chain of phenylalanine and that of chrysin. Whereas, Arg394 formed hydrogen bond with 3-OH of 17β-estradiol, Leu428, 384, 349 Phe425, Gly521, Met421, 528, 343 formed Vander Waals, Leu391, Met388, Ile424, Leu346, 387, Ala350 were involved

in pi-alkyl with aromatic π bond of 17β-estradiol and alkyl side chain the amino acid involved, Leu525 was linked to alkyl interaction with methyl group (C18) of 17β-estradiol, Phe404 formed π –π interaction. While Glu353 and His524 formed unfavorable clashes with 17β-estradiol in alpha receptor.

However, with beta receptor, chrysin formed Vander Waals interactions with Met336, 340, 295, Leu301, 380, Glu305, Arg346, Phe356, 377, Ile376, 373, His475, Gly472. And formed pi-alkyl with π bond of aromatic ring of chrysin and alkyl side chain of Leu298, 343, 339, Ala302. In addition, Glu305, Thr299, Met336, 295, Leu476, 380, 298, 343, His475, Gly472, Ile376, Arg346 involved in Vander Waals interaction with 17β-estradiol. While Ala302, Phe356, 377, Ile373, Leu298, 339, 301, Met340 was linked to pi-alkyl interaction with aromatic π bond and alkyl side chain. These favorable interactions between the molecules were point out by the very low binding energies and high inhibition binding constants (Table 1).The docked complexes were ranked based on geometric shape complementary score due to optimal fit with wide interface area and lesser stearic clashes. The ligand fit into the active site of both alpha and beta estrogen receptors with hydrogen and hydrophobic interface.

DISCUSSION

Functional foods are classify as food in which besides their natural nutritional values, exhibits health-promoting properties or prevent diseases in humans (Betoret *et al.,* 2011). Therefore, natural or synthetic compounds capable of binding and enhancing estrogen receptor sensitivity and functions could be useful ingredient in the formulation of nutraceutical agent against postmenopausal osteoporosis. Estrogen plays a crucial role in skeletal growth and bone homeostasis and has been shown to directly stimulate bone formation via enhancement of osteoblast adhesion to the extracellular matrix and stabilize bone turnover (Shur *et al.,* 2007). In addition, estrogen has been implicated in bone formation via estrogen receptor–dependent mechanism (Manolagas, 2000). Moreover, this study demonstrated that the amino acid residues involved in binding of 17β-estradiol by estrogen receptors were almost same with those involved in binding of chrysin (Chelsea & William, 2015; Xie *et al*., 2007). Similarly, estrogen enhance bone formation by reducing the formation and function of osteoclast cells through inhibition of RANKL and M-CSF which are involved in osteoclastogenesis (Gallet *et al.,* 2013; Blair *et al.,* 2006; Lee *et al.,* 2005). Estrogen receptors in differentiated osteoclasts cause decrease in bone-resorbing activity and enhanced osteoclast apoptosis (Gallet *et al.,* 2013; Chen *et al.,* 2005). In mammals, the cellular responses to estrogens are mainly mediated by ER-α and ER-β which exhibits variable expression, distribution as well as distinct signaling responses (Dhananjaya *et al.,* 2012). Loss of estrogen also affects osteoblast progenitor cells through decrease in estrogen receptor-α (ER-α) expression and lower response to mechanical stimulation (O'Brien *et al.,* 2014). This study, reported that chrysin fit into the active site of both alpha and beta estrogen receptors with hydrogen and hydrophobic interface. This finding is consistent with previous study which reported that chrysin binds into the active site of protein with both hydrogen and hydrophobic interactions (Babangida *et al.,* 2018). Also previous studies revealed that phytochemical component in herbal supplements bind to human estrogen receptor and may exhibit selective estrogen receptor modulation (Muchtaridi *et al.,* 2017; Chelsea & William, 2015). This property could be attributed to the presence of hydrophobic aromatic ring and free hydroxyl group at position 5 and 7 of chrysin. These interactions probably suggested a process in which water molecules in the cavity were replaced by guest molecules via Vander Waals forces between the molecules (Mohandoss *et al.,* 2018). In addition, binding of chrysin into the active site could trigger a signal activation of estrogen receptors (Ng *et al.,* 2014). This simulation may provide an insight on the receptor binding stability and potential estrogen-like effect of chrysin in vivo (Banbangida *et al.,* 2018).

CONCLUSION

The present study demonstrated that chrysin can effectively binds to both ERs in almost same binding site with that of estrogen and could enhances the sensitivity and function of estrogen receptors**.** Altogether, chrysin have shown a greater conformational adaptability in its binding geometry within both ER-α and ER-β, thus proving that chrysin molecule could be of particular interest and may be useful ingredient in the formulation of nutraceuticals against postmenopausal osteoporosis. However, further studies especially in vitro and in vivo studies are required to validate the potential beneficial effects of chrysin against postmenopausal osteoporosis.

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CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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