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RESEARCH ARTICLE

Potential of Biological Activities from Chemical Compounds of Nigella Sativa as Anti-Osteoporosis, an In-Silico Study

Mohammad Kuntadi Syamsul Hidayat[1,2*], Kusworini[3], Achmad Rudijanto[4], Sutiman Bambang Sumitro[5]

1. *Faculty of Medicine, University of Brawijaya Malang, East Java, Indonesia.*
2. *Doctoral Program of Medical Science, University of Brawijaya Malang, East Java, Indonesia.*
3. *Department of Clinical Pathology, Faculty of Medicine, University of Brawijaya, Malang, Saiful Anwar Public Hospital, Malang, East Java, Indonesia.*
4. *Department of Internal Medicine, Faculty of Medicine, University of Brawijaya, Malang, Saiful Anwar Public Hospital, Malang, East Java, Indonesia.*
5. *Department of Biology Faculty of Mathematics and Natural Sciences, University of Brawijaya Malang, East Java, Indonesia.*

***Corresponding Address: Mohammad Kuntadi Syamsul Hidayat**

Abstract

Objective: The objective of the study was to investigate the potential of biological activities from chemical compounds of *Nigella sativa* (NS) as anti-osteoporosis using the computational chemistry Insilco study. Methods: Database search from KnapSack was conducted to find out NS active compounds. Next, explore using the PASS Server database to find out compounds that have anti-osteoporosis potential. Exploration with STITCH to analyze the interaction of NS active compounds with cell proteins related to anti-osteoporosis, then docking using PyRx to determine the bond affinity that occurs between NS active compounds and cell proteins. Result: There were 36 active NS compounds, 28 of which had anti-osteoporosis potential. One active compound that has a probability to be active value (Pa)> 0.7 and 21 active compounds that have Pa: 0.3 - 0.7. Found 6 active compounds that have interactions and play a major role in the anti-osteoporosis process. With the docking process, binding affinity longifolene is obtained with estrogen receptors: -8.2 kcal / mol compared to Bazedoxifene -9.6 kcal / mol Conclusion: From the study of insilico, *Nigella sativa* has the potential as an anti-osteoporosis

Keywords: *Osteoporosis, Nigella sativa, insilico, anti-osteoporosis.*

Introduction

The National Osteoporosis Foundation (NOF), defines osteoporosis as a disease characterized by low bone mass and structural damage to bone tissue which results in bone fragility and increases the risk of fracture, especially *hip, spine, wrist* and the others [1-2]. Osteoporosis is referred as the "silent disease" because the sufferer does not feel being attacked by osteoporosis, until experience a fracture with minimal trauma [3].

One of the pathogenesis of post-menopausal osteoporosis is decreased of estrogen concentration (hypoestrogen). Estrogen is responsible for maintaining the balance of osteoblast and osteoclast activity [4]. The decrease of estrogen results in the secretion of various cytokines, such as IL 1, IL-6 and TNFα. These cytokines stimulate osteoclast activation, resulting in osteoclastogenesis. The most important cytokines associated with *estrogen deficiency-induced bone loss* is produced by *bone marrow T lymphocytes* [5].Osteoporosis therapy has been developed for a long time. Various studies continue to be carried out to obtain ideal preparations for overcoming osteoporosis.

The history of osteoporosis therapy preparations is recommended by Food *and Drud Administration* (FDA) starting from hormone replacement therapy (HRT) (1986), calcitonin (1991), oral biphosphonat (1995),

raloxifene (1999), *teriparatide* (2002), *strontium ranelate* (2004), intravenous biphosphonat (2006/2007), Denosumab (2010), and that are still being developed are anti-sclerostin and capthesin K inhibitors [6].Many side effects arise from existing osteoporosis therapy preparations. The emergence of various side effects of osteoporosis drugs, increasingly encourage research to obtain safer drug preparations, high effectiveness but slight side effects [6-7]. Herbal medicines are interestingly to study, because they are known to have good therapeutic effects with little side effects. Research on herbal ingredients is expected to provide an opportunity as an anti-osteoporosis drug. Nigela sativa (NS) is a type of spice that is widely used in traditional medicine and as a complementary food in the Middle East.

Several studies have shown that NS has estrogenic effects [8-11]. *Nigela sativa* contains a number of anti-oxidants, including *Thymoquinon* (TQ), carvacrol, t-anethol and 4-terpinol. *Thymoquinon* is a potent antioxidant that can indirectly decrease the production of reactive oxygen species (ROS) and inhibit lipid peroxidation [12]. *Thymoquinon* is also effective to remove superoxide-ROS- which plays an important role in activating osteoclasts [13]. *Thymoquinon*and NS seeds have also anti-inflammatory effect. [14].

Methods

Exploration of Potential for Biological Activity of Chemical Compounds N. *sativa*

Exploration using a database from KnapSack (http://kanaya.naist.jp/ knapsack_jsp/top.html was done to find out the active compounds of NS. Furthermore, tracing and exploration are carried out to find out compounds that have potential anti-osteoporosis. This search and exploration was done using the PASS Server database. The PASS server is a database that contains data about compound activity in each terminology needed. Furthermore, to find out the highest antioxidant potential can be known through the PhytoChem database (http://www.phytochemindia.com/).

Analysis of N. *sativa* with cell proteins related to antiosteoporosis

STITCH networking analysis was carried out to analyze the interaction of NS active compounds with cell proteins related to anti-osteoporosis. These tools integrated chemical compounds and proteins based on published paper databases [15]

Docking Analysis

Molecular docking was done with calcitonin receptor (CALCR) (GDP ID 5II0) and estrogen receptor (ER) (GDP ID 4XI3) to determine the bonds that occurred between active compounds of NS and proteins related to osteoclast differentiation and RANKL signaling pathway. Specific docking was carried out using PyRx and directed towards the active side of CALCR and ER. The docking process used vina autodock and was carried out specifically on the active side of the target protein.

Molecular docking can predict binding affinity of a compound to certain target proteins [16]. A drug's ability to affect a given receptor is related to the drug's affinity. The higher the affinity associated with the better the potential of the drug a high binding affinity of the receptor, closely related with greater potential of the drug. Molecular docking can be used to study the mechanism of inhibition / activation of a compound against certain target proteins. Results of complex docking were analyzed further regarding amino acids involved in interactions using the LIGPLOT + program and all visualization processes using Discovery Studio visualizer. Thus, the presentation of data will be more representative and interesting [17-18].

Result and Discussions

Potential activity of N. sativa Active Compounds in the Process of Osteoporosis

It was found 36 active compounds of NS from a data base search from KnapSack and some of them were potentially to be anti-osteoporosis. The results of exploration using KnapSack showed that the active compounds of NS had high potential in the treatment of osteoporosis. The main potential of NS active compounds was calcium regulator, bone disease treatment and anti-osteoporosis.

The active compound had good potential as an anti-osteoporosis by acting as a bone formation stimulant factor and possibly also play a role in estrogen agonist. Further exploration uses the PASS Server database to

find out active compounds that have potential as anti-osteoporosis. The approach used by Pass Server was a structural approach.

The server would compare the input compounds with compounds that have been shown to have certain activities. The assessment was by measuring the value of Probability to be active (Pa) of the active compound. Probability to be active (Pa) is a value that describes the ability of an active compound in certain biological processes.

The accuracy of the potential of the active compound was assessed by looking at its Pa value. The higher the Pa value indicated more similar structure and function of active compound. This means an active compound in NS has more potential. The Pa value of more than 0.7 indicates the similarity of the input compound with the database is high, so it is probable that the compound has biological activity if tested in a laboratory. The compound has computational potential if Pa value is less than 0.7, but more than 0.3, because there are similarities in structure with compounds that have been recorded in the database, and need further laboratory testing.There were 28 active compounds which had anti-osteoporosis potential from the 36 active compounds that found in NS.

Prediction scores provided are accurate and close to the results in vitro and in vivo if the value of Pa is more than 0.7. It was found that there was 1 active compound which had Pa more than 0.7 and 21 active compounds which had Pa 0.3-0.7.

Table 1: Probability to be Active of active compounds of N.sativa

No	Metabolites	Anti-osteoporotic	Estrogen agonist	Calcium regulator	Bone formation stimulant	Bone disease treatment
1	Thymol	0.24	0.215	0.38*		0.271
2	Carvacrol	0.35*	0.248	0.407*		0.381*
3	alpha-Thujene	0.497*	0.125	0.503*		0.511*
4	alpha-Pinene	0.221	0.203		0.237	0.212
5	beta-Pinene	0.589*	0.312*	0.555*	0.377*	0.588*
6	Myrcene					
7	Lauric acid	0.229		0.458*		
8	Oleic acid	0.18		0.477*	0.295	0.222
9	Anisaldehyde	0.263	0.168			0.238
10	Apiol			0.279		
11	Estragol	0.167		0.3*		0.179
12	Myristicin			0.37*		
13	(+)-R-Citronellol			0.426*	0.233	0.202
14	p-Cymene		0.099			0.264
15	(+)-Fenchone	0.327*	0.164	0.369*	0.151	0.331*
16	alpha-Phellandrene			0.365*		0.151
17	gamma-Terpinene		0.339*	0.389*		
18	Longifolene	0.59*	0.357*	0.531*	0.241	0.61*
19	(Z,Z,Z)-Octadeca-9,12,15-trienoic acid	0.174		0.48*		
20	Thymoquinone		0.087	0.328*	0.315*	0.213
21	Kaempferol 3-glucosyl-(1->2)-galactosyl-(1->2)-glucoside		0.27	0.338*		
22	Quercetin 3-glucosyl-(1->2)-galactosyl-(1->2)-glucoside		0.272	0.388*		
23	Quercetin 3-(6""-feruloylglucosyl)-(1->2)-galactosyl-(1->2)-glucoside		0.187	0.363*		
24	Salfredin B11		0.159			
25	Fuzitine					
26	Nigellicine					
27	Nigellidine					
28	Nigellimine					
29	4-Terpineol	0.346*	0.116	0.455*		0.353*
30	4(10)-Thujene	0.743*	0.167	0.708*	0.37*	0.782*
31	Nigeglanine					
32	Nonane			0.435*	0.191	
33	Carvone					
34	alpha-Longipinene	0.245	0.216	0.369*		0.254
35	Dihydrocarvone					
36	Nigellidine 4-O-sulfite		0.173			

*Pa > 0.3

Analysis of N. sativa with Cell Proteins Related to Anti-osteoporosis

STITCH was used to find the interaction of active compounds of NS with cell proteins related to anti-osteoporosis (TRAF 6, NFATc 1 and Chaptesin K). The results showed that there were 6 active compounds from NS that had interactions and play a major role in the anti-osteoporosis process: myristicin, thymoquinone, thymol, kaempferol, quercetin and lauric acid. There are 2 mechanisms that are affected, osteoclast differentiation and bone resorption (Figure 1).

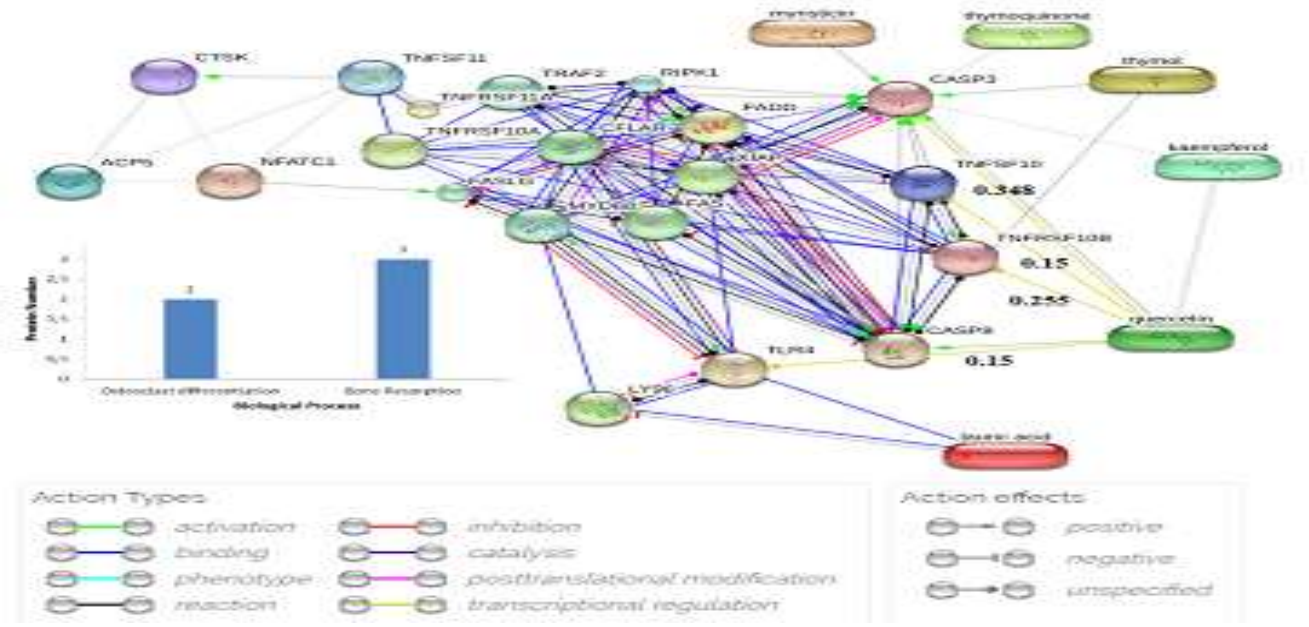

Figure 1: Pathway analysis of the active compounds *Nigella sativa* and cell proteins related to anti-osteoporosis

Until now, the direct relationship between NS active compounds in increasing expression or activation of cell proteins associated with anti-osteoporosis (TRAF6, NFATC1, and CTSK / Cathepsin K) was not known (indicated by gray lines in the Figure 1). This was due to the limited study of NS into those proteins. It showed that this study had a novelty that has further urgency to study. The results of pathway analysis showed that this active compound had a direct effect on the activity of several proteins, such as TLR4, CASP3, CASP8, and LY96. Proteins that were highly influenced and had an important role were TNFSF11 (RANKL) (Figure 2). RANKL protein had an important role because this protein had many interactions with other proteins and activates transcription factors that function in improving osteoporosis. [19-21].

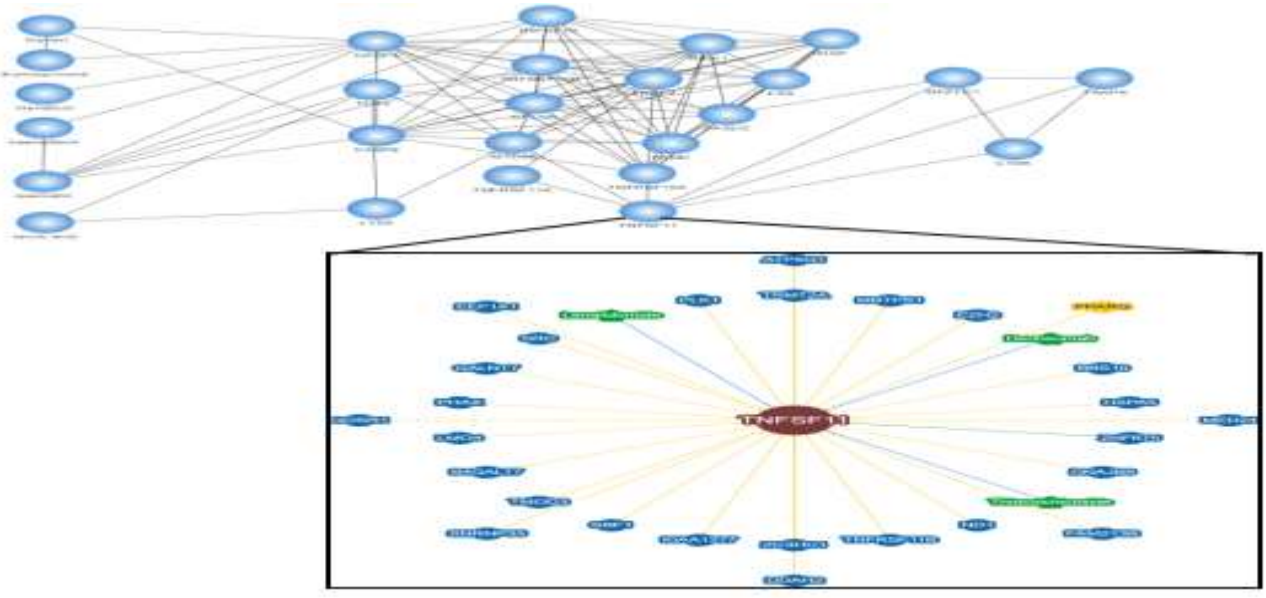

Figure 2: Interaction of TNFSF11 protein (RANKL) with other proteins involved in the mechanism of repair of osteoporosis

Analysis of Molekular Docking of Calcitonin Reseptor and Nigella Sativa Bioactive Compounds

Calcitonin receptor (CALCR) plays a role in the process of bone resorption which is important for osteoclast formation in bone remodeling. In its action, CALCR is inhibited by calcitonin (CL) as a ligand, which if not balanced will delay the osteoporosis process [22]. In addition, an important component that affects osteoporosis, more specifically in bone mineral density is estrogen level. The bond between estrogen (E) and estrogen receptor (ER) can inhibit the process of osteoporosis [23]. To determine the role of NS active compounds in the postponement of the osteoporosis process such as CL and E ligands, an affinity bond comparison was performed with each of the receptors: CALCR and ER. The results showed that the active compound NS-Quercetin3-(6""-*feruloylglucosyl*)-(1->2)-*galactosyl*-(1->2)-*glucoside* (ID 44259309)-had the potential to bind to CALCR on its activity site, with binding affinity of -8.4 kcal / mol, while analog calcitonin has a bond of -11 kcal / mol (Table 2).

Table 2: Visualize the interaction of calcitonin analogue-CALCR (A) and bioactive compounds of *N. sativa* and CALCR (B) and the amino acids involved.

Ligand	Reseptor	Binding Affinity (kcal/mol)
salmon calcitonin analogue (kontrol)	Calcitonin reseptor	-11
Quercetin 3-(6""-feruloylglucosyl)-(1->2)-galactosyl-(1->2)-glucoside	Calcitonin reseptor	-8.4

The interaction formed between calcitonin analogue and CALCR involved THR75, TRP76, GLY8, ASP77, and THR127. Whereas the Quercetin3-(6""-feruloylglucosyl)- (1->2)-galactosyl- (1->2)-glucoside and CALCR involved the amino acid TRP79, HIS121, GLU123, SER129, THR127,TRP128, TYR131, and ASN135 (Figure 3).

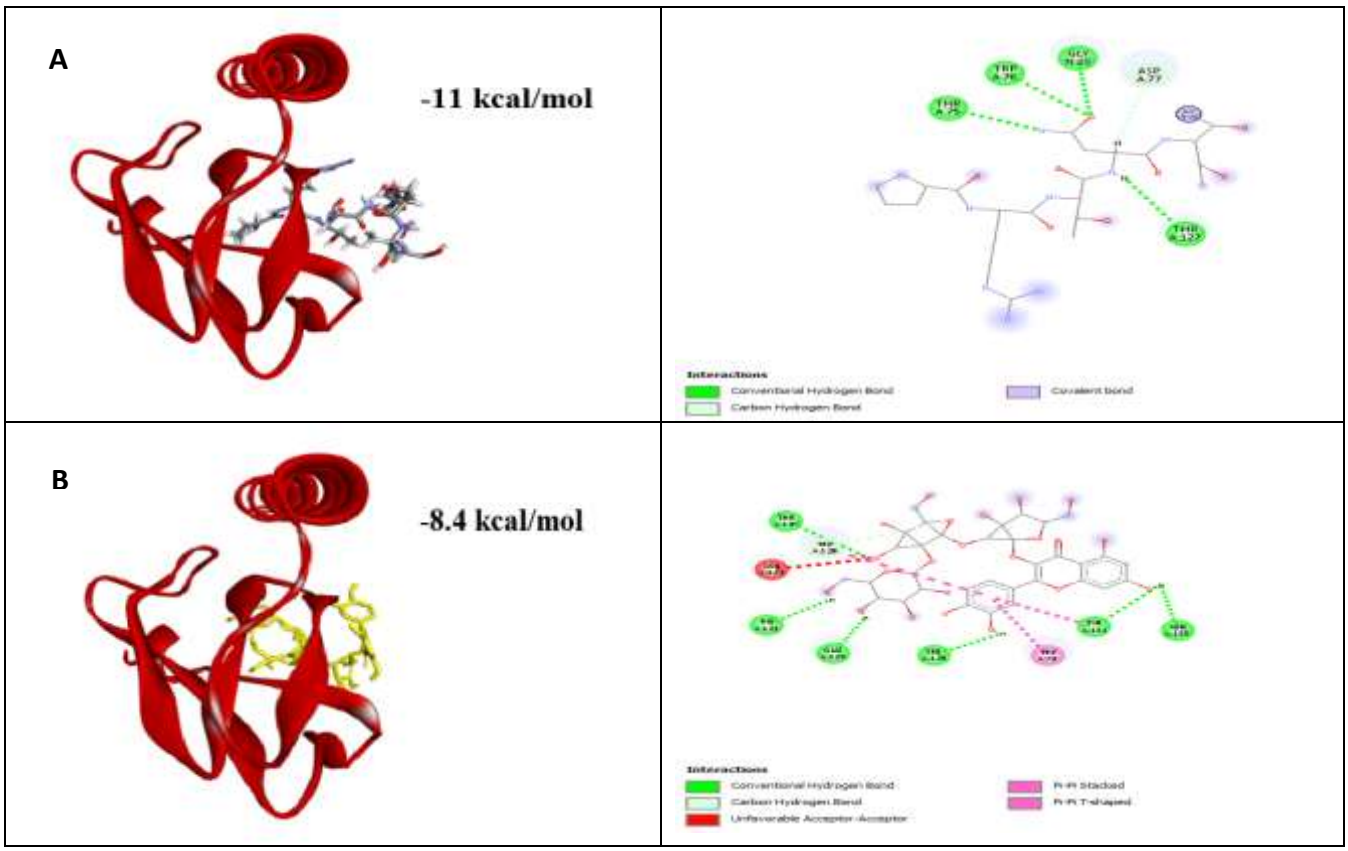

Fig. 3: Comparison of binding affinity interactions between active compounds N. sativa and CALCR

The interaction between longifolene (an active compounds of NS) and ESR showed a potential bond similar to Bazedoxifene as a control (-9.6 kcal / mol), with affinity bindings of -8.2 kcal / mol. The interaction of longifolene and ESR involved LEU346, TRP383, ALA350, LEU525, LEU384, MET343, LEU391, LEU428, MET421, and

LEU387, while BZA and ESR involved ASP351, CYS530, LEU536, TYR526, LYS529, LEU525, ALA350, LEU346, LEU346 (Figure 3, Table 3).

Table 3: Comparison of binding affinity interactions between active compounds N. sativa (Longifolene) and ESR

Ligand	Reseptor	Binding Affinity (kcal/mol)
Bazedoxifene (kontrol)	Estrogen Receptor Alpha	-9.6
Longifolene	Estrogen Receptor Alpha	-8.2

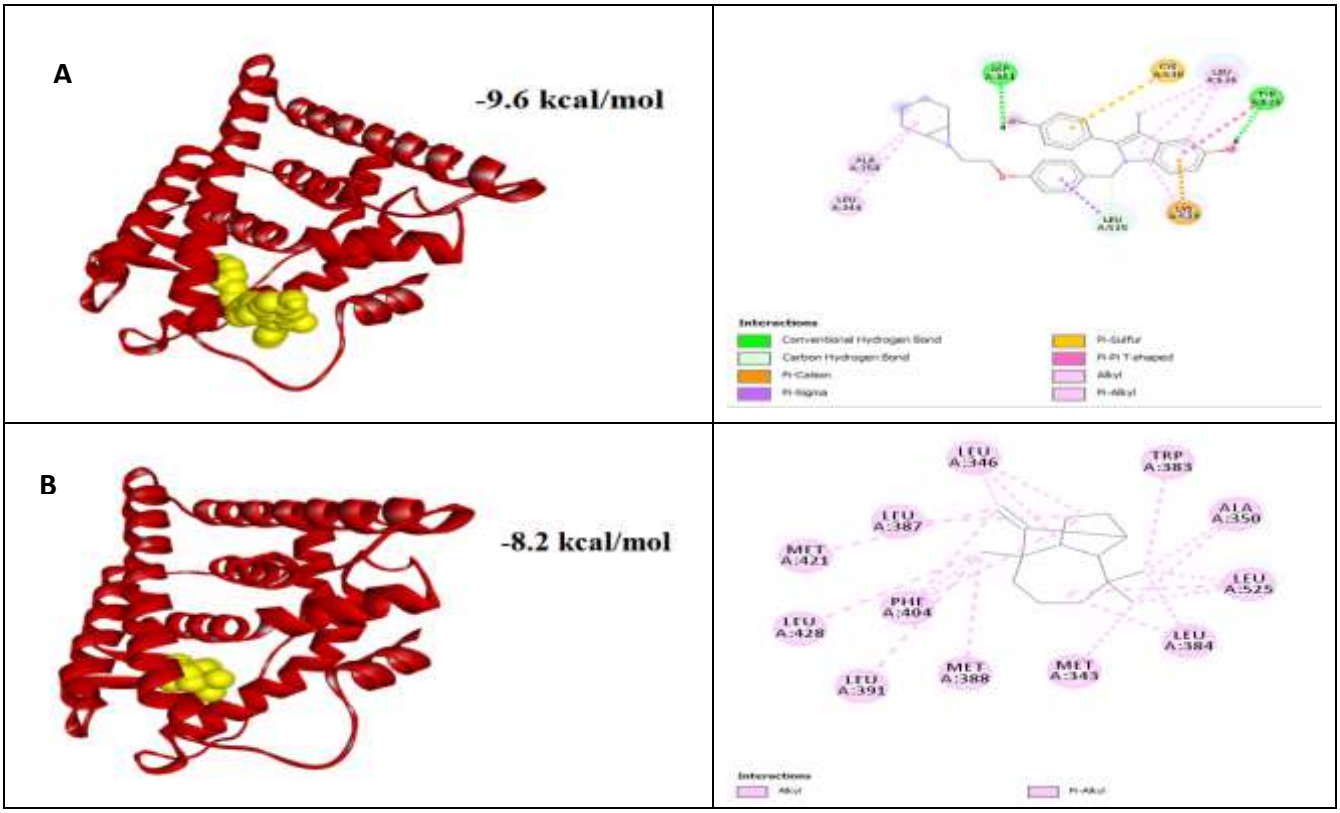

Fig 4: Visualization of the interactions of BZA-ER (A) and bioactive compounds N. sativa and ER (B) and the amino acids involved

Based on the docking analysis, it can be concluded that the active compounds of NS have the potential to inhibit the process of osteoporosis by binding to CALCR and ER as a delay in the process of osteoporosis.

Conclusion

From the study of insilico N. sativa had potential as anti-osteoporosis because there are similarities in structure with compounds that have been recorded in the database. Thus, it is feasible to carry out further laboratory tests to prove the potential of NS as anti-osteoporosis empirically.

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