S.N. Yusharyahya et al /J. Pharm. Sci. & Res. Vol. 11(12), 2019, 3705-3711

ISSN:0975-1459
Journal of Pharmaceutical Sciences and Research
www.jpsr.pharmainfo.in

In silico molecular docking and pharmacophore modelling studies of Trigonella foenum-graceum (fenugreek) interactions with estrogen receptors α and β

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Abstract

Estrogen deficiency following menopause results in the acceleration of skin ageing. Oral and topical hormonal therapy may delay this process. However, the incidence of serious adverse events raises a concern about the safety of those drugs. Phytoestrogen is thought to be a safer alternative. Fenugreek (*Trigonella foenum-graceum)* contains steroidal sapogenins, alkaloid, flavonoid (orientin) and galactomannan. Although they may bind to estrogen receptors (ER), it is unclear whether they have synergistic or antagonistic effects. We developed a computational model to identify the physicochemical characteristics of a new drug. AutoDockTools was used to predict the most active compound of fenugreek and its possible binding site to ERα and ERβ. Its affinities were compared to 17-β estradiol and tamoxifen. The active ligands were identified by pharmacophore modeling using LigandScout 4.09.1. Based on in silico study, orientin had the lowest ΔG and Ki values compared to 17-β estradiol and tamoxifen. Orientin and galactomannan showed similar features according to pharmacophore fit results and modeling. In conclusion, orientin and galactomannan perform the best synergistic activity with ERα and ERβ.

Keywords: docking, estrogen receptor, fenugreek, in silico, pharmacophore, phytoestrogen

INTRODUCTION

Skin is the largest and outermost organ in the human body. There are three principal components involved in maintaining the structural integrity of the skin, the collagen fibres, the elastic fibres, and the glycosaminoglycans [1].

Menopause, the natural end of female reproductive cycle is usually defined as the permanent cessation of menstruation resulting from a complex phenomenon involving many endocrinological changes, followed by the onset of the loss of ovarian follicular activity [2]. The menopause causes hypoestrogenism and accelerating age-related skin deterioration, such as in thinner skin due to atrophy of skin layers, an increase in number and depth of wrinkles, xerosis, and reduced skin firmness and elasticity [3].

There are many modalities used to slow the process of skin ageing, but the gold standard for topical therapy is tretinoin/retinoic acid. However, tretinoin/retinoic acid often induces irritation, dryness, redness, swelling, and photosensitivity. Estrogen as hormone therapy (HT) can also be an option as it has shown to be useful for improving the appearance of ageing skin by increasing collagen content, elastin, sebum production, improves turgor, hydration, skin thickness, and decrease wrinkling [4]. However, various reports on its severe side effects such as increased risk of breast cancer, stroke and cardiovascular diseases have raised a serious concern over the use of HT purely for the treatment of skin ageing [3].

Various reports on side effects of HT have generated an increasing interest in the development of safe natural agents for the management of postmenopausal discomforts. It has been widely studied that certain plant-derived natural compounds contain estrogenic effects (phytoestrogens) [5]. These compounds can be used for the management of menopausal symptoms due to their selective estrogen receptor modulating (SERM) properties. *Trigonella foenum-graceum* (fenugreek) is one of the phytoestrogens available on the market.

Fenugreek is a dicotyledonous plant, belonging to the family of Leguminosae/Fabaceae [6]. Fenugreek is cultivated as vegetable and spice crop for a long time all around the world, especially in Iran, India, Egypt; and is now being cultivated in Indonesia (Lembang) as well. The seeds and leaves are consumed all around the world for both medicinal and non-medicinal purposes. It is believed to have anti-inflammatory, antioxidant, and can increase milk supply in breastfeeding mother [7]. Fenugreek has also performed an antidiabetic activity due to a huge amount of galactomannan fibre in its seeds [8].

Fenugreek is a natural source of iron, sodium, thiamine. The primary biologically active ingredients of fenugreek are steroidal sapogenins (diosgenin, yamogenin, gitogenin, tigogenin), alkaloid (trigonelline), flavonoid (quercetin, vitexin, isovitexin, orientin, isoorientin) and a non-proteinogenic amino acid '4-hydroxyisoleucine' [6, 7, 9]. Some ingredients i.e., trigonelline and 4-hydroxyisoleucine, are believed to able to bind to the estrogen receptors [10]. However, among those biologically active components, it is not yet known which compound can activate or inhibit estrogenic effect, and which compound is the most potent compound promoting those effects.

Computational (in silico/molecular docking) methods have been developed and widely applied in biomolecular and biotechnological field for pharmacology hypothesis

development and testing. It is also a computational procedure that attempts to predict the efficiency of non-covalent binding between macromolecules (receptor) and small molecule (ligand) [11]. These methods are instrumental in discovering novel molecules with affinity to target receptors, absorption, distribution, metabolism, and excretion. All of which are useful to identify physiochemical characterization of a new drug [12]. In this study, we developed a computational model using Autodock tools program and pharmacophore models to predict possible binding sites between *Trigonella foenum-graceum* (Fenugreek) to estrogen receptors α and β.

METHODS

All computational methods were conducted with a Macbook of Mac OS High Sierra v10.13.3 operating system, with processor 1,8 GHz Intel Core i5, RAM 8 GB, 1600 MHz DDR3, Graphics Intel HD 6000 1536 MB.

Protein preparation

Data on protein estrogen alpha and beta were obtained from NCBI (estrogen alpha receptor: https://www.ncbi.nlm.nih.gov/protein/P03372.2; and estrogen beta receptor: https://www.ncbi.nlm.nih.gov/protein/Q92731.2) and www.rcsb.org in a .pdb file format. Data on the crystal structure of estrogen receptor alpha (PDB ID: 4ZN7) and beta (PDB ID: 1U3Q) were obtained from protein data bank (http://www.pdb.org/). The macromolecule was then optimized using Autodock tools to fix the charge, add the hydrogen, merge the nonpolar, and minimize the energy. The structure was then saved in .pdbqt format.

Ligand preparation

Native ligand from PDB (PDB ID: 4ZN7) and beta (PDB ID: 1U3Q) was separated from the ligand-receptor complex. The structure of ligand compounds from *Trigonella foenum-graceum* (Fenugreek) was made in 2D using Marvin Sketch 15.1.19 software and saved in 3D structure in .pdb format [2].

Interaction between molecular docking and ligand-receptor

Molecular docking of designed compounds was carried out with Lamarckian genetic algorithm default in Autodock 4.2 tools. We selected the Autodock 4.2 tool for molecular docking because Autodock is a useful tool that can accurately predict bound conformations and binding energies of ligands with macromolecular targets. We used 17-ß estradiol and tamoxifen (SERM) as positive controls. The reason for using two positive controls is to know the mechanism of the Fenugreek seeds' compound bonding compared to the mechanisms of the positive control bond.

Docking method validation

Docking was conducted on native ligand PDB by first optimizing the size of the grid box and grid centre position. The optimal size of the grid box and grid centre were chosen by evaluating the results of docking interactions. (Grid box coordinate (x = 15.609; y = 28.886; z = 83.69), point distance 0,375 Å with 40 x 40 x 40 dimension).

Docking and docking interaction analysis

Docking interactions were clustered to determine the Gibbs energy (ΔG) as the lower ΔG shows the conformation energy of the best-docked value.[11] An estimated concentration of inhibitor (Ki) was reported to determine the binding energy which was produced from the docking. Each compound has different conformations in correlation to the value of binding energy.

To conduct and analyse the docking interactions, we input the receptors and ligands. Then we made a grid file by adjusting the grid box and grid centre with an output file format of .gpf. Then, docking file was made by adjusting the docking parameter with an output file format of .dpf. We ran the autogrid and autodock command. The complete docking process in a .dlg file format was sent to Autodock software to analyse the docking, Ki value as well as visualize receptor-ligand complex from the docking process.

Pharmacophore model

The step in the development of the pharmacophore model is an active selection of ligands known to be tied to the same ER - α (same binding area). Then, we conduct conformation analysis to know the standard 3D pharmacophore features and to determine the Query Pharmacophore. The development of 3D pharmacophore model uses LigandScout 4.09.1 (ligand-based) with the preparation of ligand by collecting the known active compound files (in. pdb format,. mol or. SMI) beforehand. Afterward, we form ligand-set conformation and pharmacophore 3D model using a training set [13].

Virtual Screening

Virtual screening starts by downloading some compounds from ZINC databases and are saved as a database with .mol format. Best pharmacophore model is chosen to undergo a screening process. Furthermore, the database is inserted into "screening databases" and marked with green colour. One pharmacophore model is selected, and the screening process is conducted by clicking the "Perform Screening" icon. This step is repeated with another pharmacophore model. The results of hit compound fenugreek screening are sorted based on the best Pharmacophore fit score value. Visualization of pharmacophore features hit compounds include HBA (Hydrogen Bond Acceptor), HBD (Hydrogen Bond Donor), AR (Aromatic Ring), Hydrophobic Interactions (HI) [13].

RESULTS

The chemical binding compounds of Fenugreek seeds found by docking are known to contain several biologically active ingredients such as alkaloids (trigonelline), sapogenin steroids (diosgenin, yamogenin, gitogenin, tigogenin), polysaccharides (galactomannan), 4-hydroxyisoleusin, flavonoids (quercetin, vitexin, isovitexin, orientin, isoorientin).

We conducted a molecular docking study for twelve alkaloid compounds from Fenugreek seed which were estimated to be able to bind to estrogen receptors-alpha (ER-α) and -beta (ER-β) and also added 17-β estradiol and

tamoxifen as controls. The binding energies (ΔG), inhibitor concentration (Ki), hydrogen bond (Hbond) involved in the ligand-receptor complex formation were determined.

Bonding of Fenugreek compounds with estrogen receptor alpha (ER-α)

The twelve alkaloid compounds showed successful docking inside the active site of ER-α (see fig. 1) with the binding energy of -3.79 to -10.17 Kcal/mol, as well as 17-β estradiol and tamoxifen with the binding energy of -10.01 Kcal/mol and -10.00 Kcal/mol, respectively (see table 1.).

The twelve compounds bound to estrogen receptors with 1 hydrogen bond, while estradiol showed 2 hydrogen bonds and tamoxifen had 1 hydrogen bond (see table 1.). In addition, orientin revealed the lowest ΔG and Ki values, and even lower compared to ΔG and Ki values of estradiol and tamoxifen, while 4-hydroxyisoleucine showed the highest ΔG and Ki values compared to other eleven alkaloids compounds and controls (see table 1.).

Based on PDB, the binding sites of ERα are Thr343, Ala350, Glu353, Leu387, Arg394, Gly521, His524, and Met528. The data are obtained from http://www.rcsb.org/pdb/explore/remediatedSequence.do?structureId=4ZN7. Based on docking simulation orientin had the lowest ΔG and Ki values, even lower compared to 17-β estradiol and tamoxifen, which means that this molecule is most active when bound to estrogen receptors α. The hydrogen bonds that occur between the orientin and the receptor were similar to the hydrogen bonds that were present between 17-β estradiol and its receptor, called His524. The only difference was there were no hydrogen bonds in Arg394 of orientin. Besides, Quercetin (which is also a class of flavonoids) had ΔG and Ki values closer to 17-β estradiol and contained hydrogen bonds in His524 receptor. Galactomannan, which is a class of polysaccharides, also had ΔG and Ki values similar to 17-β estradiol and hydrogen bond in His524 receptor. Therefore, the mechanism of action of orientin, quercetin, and galactomannan were thought to be similar (synergistic) to the mechanism of action between 17-β estradiol and estrogen receptors α.

Bonding of Fenugreeks compounds with estrogen receptor beta (ER-β)

We observed that all twelve compounds successfully bind to the active site of ER-ß with positive and negative binding energy variations, while all controls, 17-ß estradiol, and tamoxifen revealed negative energy binding to ER-ß. (see fig. 2)

Galactomannan, orientin, quercetin and 4-hydroxyisoleucine bound to ER-ß with negative energy binding of -9.79 Kcal/mol, -9.63 Kcal/mol, -9.45 Kcal/mol, -4.01 Kcal/mol, respectively. On the other hand, other alkaloid compounds bound to ER-ß with positive energy binding, ranging between +1.44 Kcal/mol to 53.42 Kcal/mol (see Table 2.)

Galactomannan showed the lowest energy binding to ER-β (-9.79 Kcal/mol) compared to other alkaloids and tamoxifen, but that binding was not lower compared to 17-ß estradiol (-10.70 Kcal/mol) (see Table 2.).

Based on PDB, the binding sites of ERβ are Met340, Ile376, Gly472, His475, and Leu476. (data from http://www.rcsb.org/pdb/explore/remediatedSequence.do?structureId=1U3Q). Based on docking simulation, orientin compounds were the most active with the smallest ΔG and Ki values, exceeding that of tamoxifen. The hydrogen bonds occurred between orientin and galactomannan, and their receptors were similar to the hydrogen bonds that existed between tamoxifen and its receptor, Glu305 and also Gly472. However, the hydrogen bonds that occurred between orientin and galactomannan and their receptors were different compared to the hydrogen bonds of 17-β estradiol and its receptors. Therefore, the mechanism of action of orientin and galactomannan were considered similar to the mechanism of action of tamoxifen, instead of 17-β estradiol.

Table 1. The Gibbs energy (ΔG), inhibitor concentration (Ki) and Hbond characteristics of fourteen Fenugreek seed compounds against ER - α (PDB ID: 4ZN7)

No	Compound	ΔG (Kcal/mol)	Ki (μM)	Hbond
1	4-hydroxyisoleucine	−3.79	1680	1 (Arg394)
2	Vitexin	−6.06	36.42	1 (Ala350)
3	Diosgenin	−7.26	4.79	-
4	Trigonelline	−7.91	1.60	1 (Thr347)
5	Isoorientin	−7.98	1.42	1 (Thr347)
6	Yamogenin	−8.12	1.11	-
7	Isovitexin	−8.13	1.10	1 (Thr347)
8	Tigogenin	−8.26	0.88	1 (Asp351)
9	Gitogenin	−8.41	0.681	1 (Asp351)
10	Galactomannan	−9.00	0.252	1 (His524)
11	Quercetin	−9.55	0.1	1 (His524)
12	Orientin	−10.17	0.035	1 (His524)
13	17-ß estradiol (+ control)	−10.01	0.046	2 (Arg394, His524)
14	Tamoxifen (+ control)	−10.00	0.047	1 (Asp351)

Table 2. The Gibbs energy (ΔG), inhibitor concentration (Ki) and Hbond characteristics of fourteen Fenugreek seed compounds against ER- β (PDB ID : 1U3Q)

No	Compound	ΔG (Kcal/mol)	Ki (μM)	Hbond
1	Galactomannan	− 9.79	0.066	2 (Leu298, Glu305)
2	Orientin	− 9.63	0.087	3 (Glu305, Leu339, Gly472)
3	Quercetin	− 9.45	0.117	1 (Arg346)
4	4-hydroxyisoleucine	− 4.01	1160	1 (Arg346)
5	Vitexin	+ 1.44	-	1 (Arg346)
6	Isovitexin	+ 7.89	-	3 (Arg346(2),His475)
7	Diosgenin	+ 8.95	-	1 (Arg346)
8	Trigonelline	+ 13.67	-	3 (Arg346(2),His475)
9	Isoorientin	+ 15.62	-	3 (Arg346(2),His475)
10	Yamogenin	+ 31.81	-	1 (His475)
11	Tigogenin	+ 39.53	-	2 (Arg346(2))
12	Gitogenin	+ 53.42	-	2 (Arg346(2))
13	17-ß estradiol (+ control)	−10.70	0.014	1 (His475)
14	Tamoxifen (+ control)	−9.44	0.121	1 (Glu305)

Table 3. Pharmacophore features of Fenugreek seed compounds with ER - α (PDB ID: 4ZN7)

No	Compounds	Pharmacophore Feature	Pharmacophore Fit
1	Diosgenin	HI (2)	33.50
2	Galactomannan	HI (3), HBD (2)	42.61
3	Orientin	HI (3), HBD (1), HBA (2)	45.22
4	Trigonelline	HI (1), HBA (1)	30.19
5	17-ß estradiol	HI (1), HBA (1), HBD (1)	30.95
6	Estradiol as the training set	HI (1), HBA (1), HBD (1)	40.38

Yellow: Hydrophobic Interaction (HI), Red: Hydrogen Bond Acceptor(HBA), Green: Hydrogen Bond Donor (HBD)

Trigonelline, vitexin, isovitexin, isoorientin had positive ΔG, in contrast to the docking results of estrogen receptors α, on β receptors, gitogenin, and all other steroidal sapogenin compounds. They all showed inhibitory function instead of activation. Majority of the compounds in Fenugreek seeds cannot be used if the goal is to utilize estrogen receptors α and β, due to non-synergistic activity of all the components on estrogen receptor, in which some showed activation, others showed inhibition.

DISCUSSION

Based on molecular docking results to the ER - α and ER-β, the results of the ER-alpha docking indicated that fenugreek extracts had a role and effect on ER-alpha. These docking results were confirmed with pharmacophore modeling as the native ligand/compound-based approach of ER-alpha. Pharmacophore fit was conducted as one of the In-Silico methods in drug discovery. This ligand-based structures approach has been developed to reveal the pharmacophore feature in compound modeling and has been successfully applied in virtual screening, de Novo design, and compounds optimization. A pharmacophore approach could reduce the costs of drug discovery and development. Pharmacophore modeling provided useful instructions for the development of estrogen therapy from fenugreek extracts. In this research, significant compounds such as galactomannan, orientin, diosgenin, and trigonelline were processed with a training set estradiol. (see fig. 3e.)

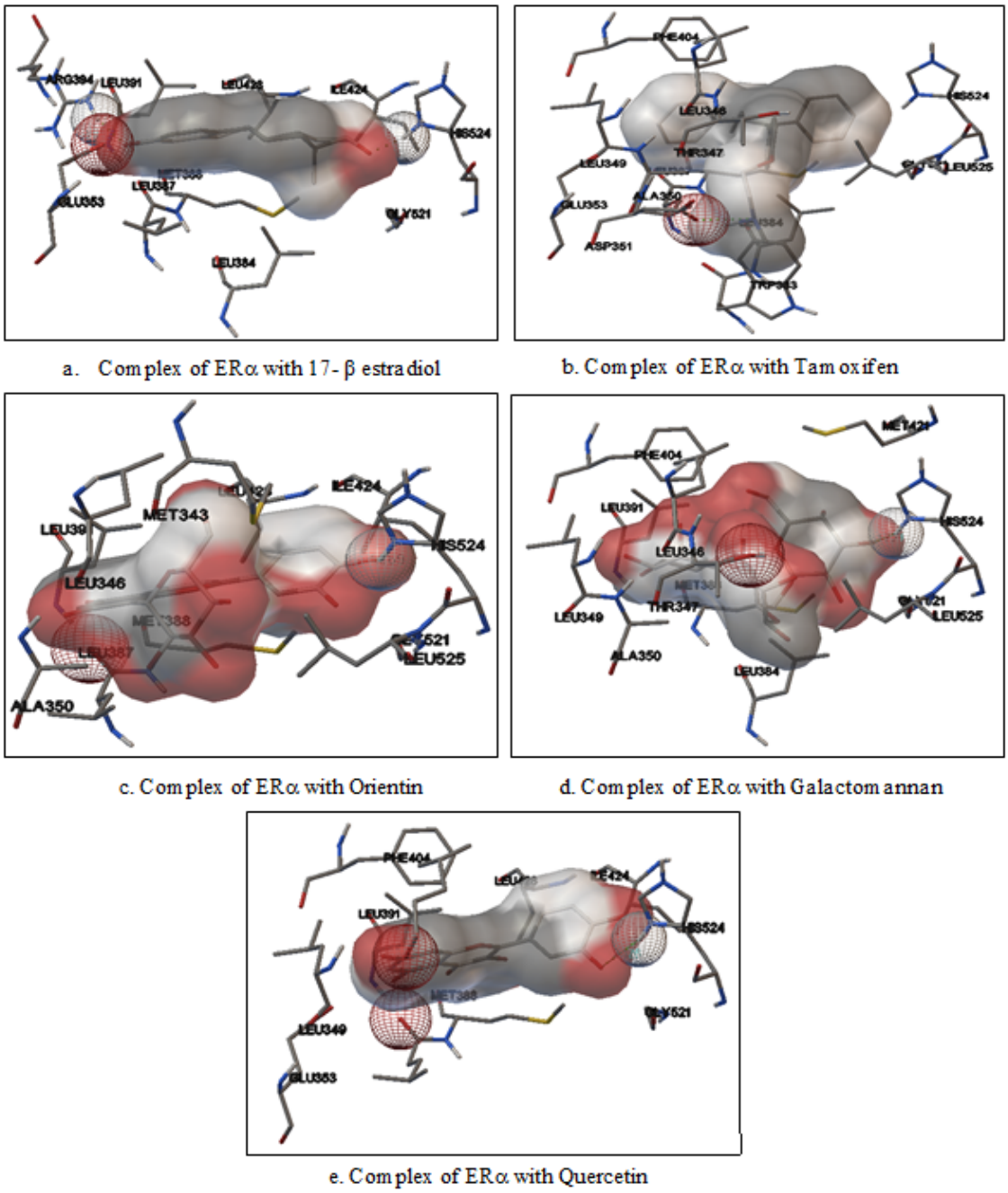
The hydroxyl group represented HBA and HBD on the Cyclopentane chain. The hydrophobicity of the training set was seen in the methyl group of the Cyclopentane chain. The pharmacophore of this training set was maintained in designing candidate compounds. The pharmacophore feature of the hydroxy group contributed to hydrogen donor and acceptor hydrogen. HBD and HBA in fenugreek can be enhanced by using extracts that contained active hydroxyl groups.

Results of pharmacophore modeling (see table 3.) showed that compounds in fenugreek extract i.e., orientin and galactomannan had similar pharmacophore feature of estradiol, i.e., HI, HBA, and HBD. Pharmacophore fit revealed the flexibility of ligand/compound conformation on the pharmacophore receptor model that was demonstrated by orientin and galactomannan with the pharmacophore fit values of 45.22 and 42.61, respectively.

Based on Pharmacophore fit value and Pharmacophore model feature, orientin and galactomannan displayed similar features. (see fig. 3a-d.) In general, the

pharmacophore multi-ligands formation consisted of two main steps: create conformation chamber for each ligand which represents the flexibility of ligands conformation, as well as, determinates standard chemical compound that is essential to construct a pharmacophore model. Pharmacophore-based ligands also include the arrangement of conformational flexibility of ligands and the conduction of molecular alignment that represents the essential techniques [12, 13].

Out of the all compounds found in fenugreek seeds, orientin had the lowest ΔG and Ki values compared to 17-β estradiol and tamoxifen based on docking. According to pharmacophore fit results and modeling, both orientin and galactomannan showed similar features. It means that orientin and galactomannan perform the best synergic activity with estrogen receptors α and β.



a. Complex of ERα with 17-β estradiol

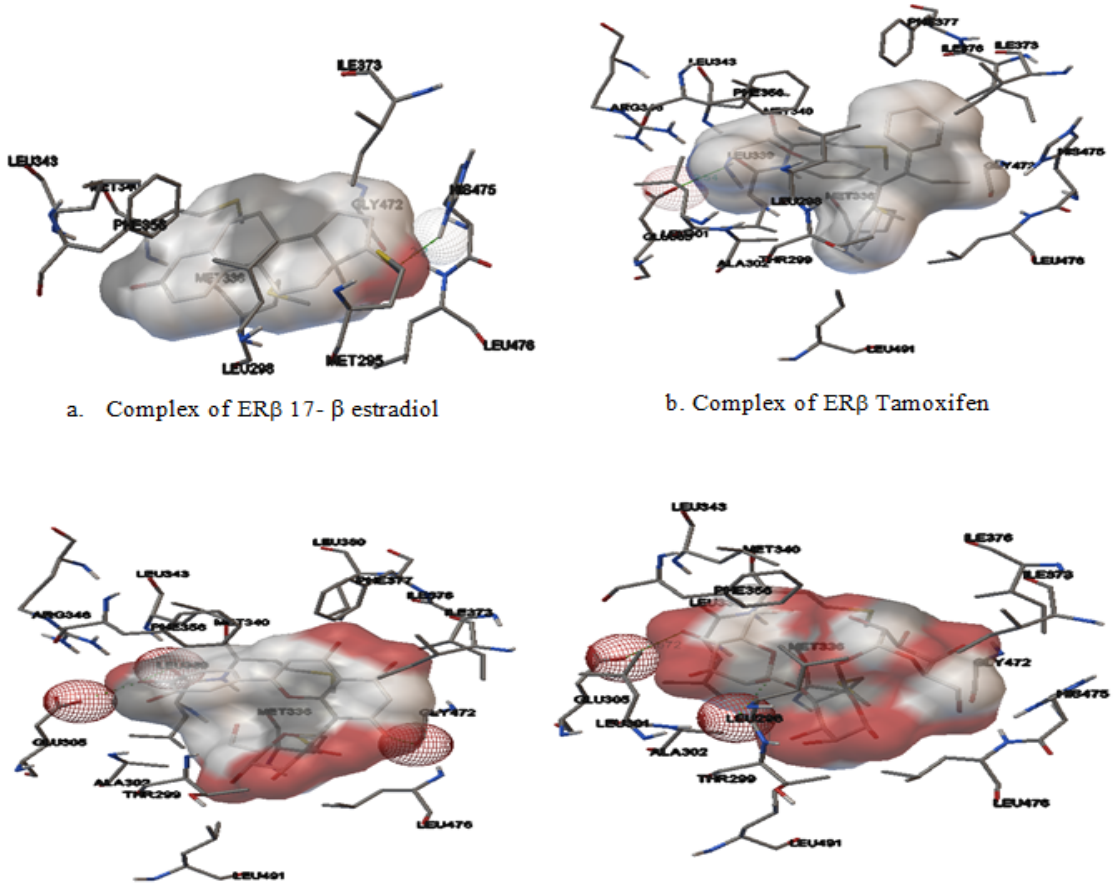
b. Complex of ERα with Tamoxifen

c. Complex of ERα with Orientin

d. Complex of ERα with Galactomannan

e. Complex of ERα with Quercetin

Fig. 1. Complex of ERα with Fenugreek seed compounds



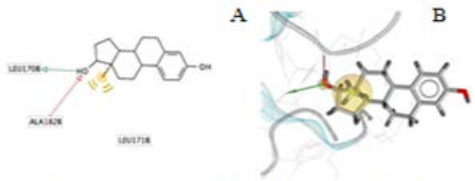
a. Complex of ERβ 17- β estradiol

b. Complex of ERβ Tamoxifen

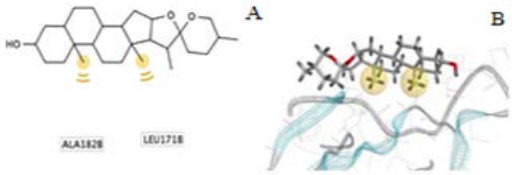
c. Complex of ERβ Orientin

d. Complex of ERβ Galactomannan

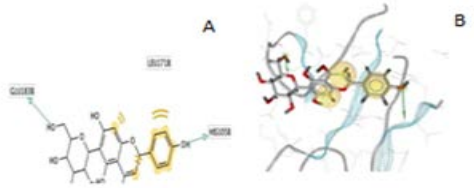
Fig. 2. Complex of ERβ with Fenugreek seed compounds



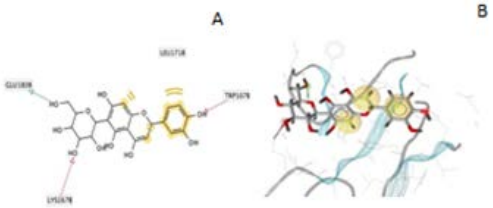
a. Pharm acophore m odel of E stra diol (A)2D



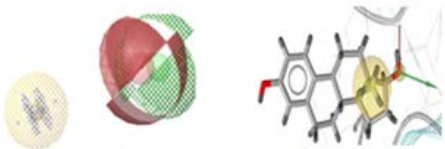
b. Pharm acophore m odel of Diosgenin (A)2D (B)3D



c. Pharm acophore m odel of Galactomannan (A) 2D (B)3D



d. Pharm acophore m odel of Orientin (A)2D (B)3D



e. 2D and 3D Pharm acophore Models with training set

Fig. 3. Pharmacophore Model

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