International Journal of Chemistry and Pharmaceutical Sciences

Journal Home Page: www.pharmaresearchlibrary.com/ijcps

Research Article **Open Access**

In-Silico Analysis of Ketosterol and It's Binding Possible Interacting Partners in Estrogen Receptor

Ravuri Venkata Rao*[1], AVVS Swamy[2], Tulasi Narahari[3], Harika Boorsu[3]

[1]*Research Scholar, Department of Biochemistry, Acharya Nagarjuna University, Nagaruna Nagar, Guntur, A.P, India*
[2]*Department of Environmental Sciences, Acharya Nagarjuna University, Nagaruna Nagar, Guntur, A.P, India*
[3]*SARC (Scientific and Applied Research Centre), Hyderabad, Telangana, India*

A B S T R A C T

The present study the bioactive compound from the bark of *Saraca indica* extracted successively using soxhlet. Hexane extract was purified and characterized by NMR studies and conformed obtained suggested that the metabolite was likely to be a Ketosterol and the molecular weight was found to be 328.0040 with molecular formula $C_{21}H_{28}0_{3.}$ The Ketosterol was subjected to molecular docking studies of Ketosterol extracted and purified from the bark of *Saraca indica* plant, Estradiol, Tamoxifen and Raloxifene with the estrogen receptor a and estrogen receptor p. This docking study proves that plant extracted ketosterol inhibits the transcriptional activation function of estrogen receptor a and p. Docking results have shown the better binding interactions compare with native ligand. Plant extracted ketosterol showed best binding affinity than default ligand which may acts as alternative to default ligand. Plant extracted ketosterol showed three hydrogen bonds with least distance than the default ligand and Estradiol, Tamoxifen and Raloxifene (SERMs) and ketosterol shows best docking energies

Keywords: *Saraca indica,* Soxhelet, NMR, Docking, Protein

A R T I C L E I N F O

CONTENTS

Article History: Received 10 September 2015, **Accepted** 29 October 2015, **Available Online** 27 November 2015

***Corresponding Author**
Ravuri Venkata Rao
Research Scholar, Dept. of Biochemistry, Acharya Nagarjuna University, Nagaruna Nagar, Guntur, Andhra Pradesh, India
Manuscript ID: IJCPS2776

PAPER-QR CODE

Citation: Ravuri Venkata Rao, *et al. In-Silico* Analysis of Ketosterol and its Binding Possible Interacting Partners in Estrogen Receptor. *Int. J. Chem, Pharm, Sci.*, 2015, 3(11): 2125-2132.

Copyright© 2015 Ravuri Venkata Rao, *et al.* This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution and reproduction in any medium, provided the original work is properly cited.

1. Introduction

Plants contain numerous biologically active compounds, many of which have been shown to have antimicrobial properties (COWANMM 1999.) Plant-derived medicines have been part of traditional health-care in most parts of the world for thousands of years and there is increasing interest in plants as sources of agents to fight microbial diseases (Chariandycm, 1999). Given the alarming incidence of antibiotic resistance in bacteria of medical importance, there is a constant need for new and effective therapeutic agents (Bhavnanism 2000, Ahmadi, 1998). *Saraca indica* (family Caesalpiniaceae) also known as *Saraca asoca* is one of the most ancient sacred plants widely distributed throughout the Indian subcontinent [24, 25]. Various medicinal uses of *Saraca indica* had been reported in Charaka Samhita (100 A.D.) [26]. Different parts of the plant exhibit a number pharmacological effects like antihyperglycemic, antipyretic, antibacterial, anthelmintic, activity, and so forth, which are well described in literature [27–30]. A traditional drug Asoka Aristha used for the treatment of menorrhagia is originated from *Saraca indica* [31]. Secondary metabolites like flavonoids, terpenoid, lignin, phenolic compounds, tannins, and so forth are reported from *Saraca indica* stem bark extracts and found responsible for their therapeutic action [32–38].

In the present study the molecular docking studies of Ketosterol extracted and purified from the bark of *Saraca indica* plant, Estradiol, Tamoxifen and Raloxifene with the estrogen receptor-a and estrogen receptor-p and also discussed dynamics and simulation studies of estrogen receptor a and p complex with ketosterol and without ketosterol to understand the time dependent behavior of the native and docked (receptor-ligand complex) structure.

A high level of Estrogen is linked with increased risk of breast cancer, which mediates its biological effects such as genesis, malignant progression, cell apoptosis and other important roles by binding to the Estrogen Receptor present in the breast cancer cells (Thomas and Gustafsson, 2011; Fatemeh et al., 2012). The Estrogen Receptor mainly exists in two forms: Estrogen Receptor alpha and Estrogen Receptor Beta. Estrogen receptors alpha (ER-α) is mainly expressed in the uterus, vagina, mammary gland, liver, pituitary gland (Waraphan et al., 2011). The major causes of breast cancer are identified by abnormal expression of Estrogen Receptor α-positive affecting about 70% of the primary breast cancer patients (Dickson and Stancel, 2000; Fuqua, 2001; Ariazi et al., 2006; Chao-Yang et al., 2013; Kumaraswamy and Suneetha, 2013)

2. Materials and Methods

Plant material

The bark of *Saraca indica* was collected from Public garden, Nampally, Hyderabad, Andhra Pradesh, India. The plant was authentified by Prof. Ram reddy, Department of Botany, Osmania University, Hyderabad, Telangana, India. The voucher specimen of the same was deposited in the herbarium of Botany Department, Osmania University, Hyderabad, Telangana, India.

Successive solvent extraction

50 g of moisture free bark powder of the *Saraca indica* was placed in a thimble-holder and 400 ml of hexane added to the solvent flask. The temperature was raised to 45°C for 16 hours (using heating mantle) and cooled to room temperature, separated the pet ether layer. Then added 400 ml of ethyl acetate and ran the soxhlet at 55°C for 16 hours. At the end, the ethyl acetate layer was separated. To the above residuary gum 400 ml of methanol was added and repeated the above procedure. Finally the extracted samples were concentrated under reduced pressure with rotary evaporator (Heidolph) to get the residues.

Characterization of bioactive compounds

The analysis of the sample was done using FTIR (recorded on JASCO FT/IR-5300- sample was prepared by dissolving 1 mg of compound in 10 micro liters of the dichloro methane (DCM), ^{1}H, ^{13}C NMR recorded on Bruker 400MHz NMR spectrometer (in CDCL3 with TMS as internal reference), DEPT, COSY and NOSY were recorded on Bruker 500 MHZ NMR spectrometer. LCMS were recorded on VG7070H mass spectrometer using EI technique or Shimadzu-LCMS-2010.LC-MS data were obtained using electrospray ionization (positive mode) on a C-18 column at a flow rate 0.2 mL/min using MeOH/water (90:10) as eluent. Further, for accurate molecular weight identification high resolution mass spectrophotometer (HRMS) technique was used. For this, sample was dissolved in methanol and submitted to the analysis.

Docking Studies:

3D-structures of ligand molecules were drawn using chemdraw software (http://www.cambridgesoft.com/). Docking was performed using GOLD software (http://gold.ccdc.cam.ac.uk), which uses genetic algorithm for finding the binding modes of the ligand. The protocol followed for docking has four main steps, a) Ligand preparation b) Receptor preparation c) Docking using a search algorithm and d) Analysis of the resultant binding modes using a scoring function as shown in flowchart. 1. Docking studies were performed on Dell inspiron 15R which is having 1TB Hard Disk, 8GB RAM, 1-7 Processor.

Selection of the receptor structures:

ER is a member of a large family of nuclear receptor (NR) transcription factors with a characteristic modular structural organization with distinct domains associated with trans activation, DNA binding and hormone binding (Tsai and Malley *et al.*, 1994). ERa and ERp share modest overall sequence identity (47%) with little homology between their N-terminal transactivation (AF-1) domains but well conserved DNA and ligand-binding domains. The C-terminal ligand-binding domain (LBD) is multifunctional and, in addition to harboring a ligand recognition site, contains regions for receptor dimerization and ligand-dependent (AF-2) transactivation (Fawell *et al.*, 1990; Danielian *et al.*, 1992). Hormone binding to ER-LBD induces a conformational change in the receptor that initiates a series of events that culminate in the activation or repression of responsive genes (Tsai and Malley *et al.*, 1994; Beato and Sanchez-Pacheco *et al.*, 1996). The precise mechanism by which ER affects gene transcription is

poorly understood but, at least in the case of AF-2 activation, appears to be mediated by umerous nuclear factors that are recruited by the DNA-bound receptor (Torchia *et al.,* 1998). The Oestrogen receptor (ER), which functions as a ligand activated transcriptional regulator (Tsai and Malley *et al.,* 1994), Oestrogens exert their physical effects via the ER which play a critical role in the growth, development and maintenance of diverse range of tissues (Pike *et al.,* 1999), these effects were attributed to a single ER. The unexpected discovery of a second ubiquitous ER, termed ERp (Kuiper *et al.,* 1996; Ogawa *et al.,* 1998), has added another layer of complexity to the inaction of oestrogens and promoted intense interest in the respective role of each isoform (Katzenellenbogen and Korach *et al.,* 1997). The two ER iso-forms exhibit overlapping but distinct tissue distribution patterns and differ in the ligand binding ability and transactivation properties (Kupier *et al.,* 1997). ER is an important pharmaceutical target for hormone replacement in menopausal women and for chemotherapeutic drugs against certain reproductive cancers. A wide range of structurally distinct compounds binds to ER with differing degrees of affinity and potency (Anstead *et al.,* 1997; Kuiper *et al.,* 1997; Barkhem *et al.,* 1998).

Ligand preparation:

Ketosterol structure was build using the Chemdraw software the molecule was minimized with the steepest descent algorithm followed by conjugate gradient algorithm from steps 500 to 1200 and were found to be stabilized at 1200 steps, estradiol at 1200 steps, Tamoxifen at 1000 steps and raloxifene at 1100 steps minimized gradient tolerance satisfied scores were displayed in table 4.1. Later molecular structures are converted to SYBYL-moI2 format which is compatible to use with the GOLD program for docking studies with Estrogen receptor a and p. The above same protocol was followed for preparation of the estradiol, Tamoxifen and, raloxifene.

Receptor preparation:

Estrogen receptor a was downloaded from the Protein Data Bank (PDB) (www.rcsb.org) database having PDB-ID: lGWR.pdb that is X-Ray crystal structure of human estrogen receptor alpha ligand-binding domain in complex with 17-beta-oestradiol and tif2 nrbox3 with resolution of 2.40 A^0 (Wammark *et al.,* 2002), and Estrogen receptor p downloaded from the PDB database having PDB-ID: lQKM.pdb that is X-Ray Crystal structure of human estrogen receptor beta ligand-binding domain in complex with partial agonist genistein with resolution of 1.8 A^0 (Pike *et al.,* 1999).

These structures were used for docking study. First, hydrogen atoms were added, second, checked the stereo chemistry of the receptor and applied the charm force field to get the structure into the geometrically stable form. Third, structure was minimized using the SYBYL-X software, molecule initially applied with steepest descendent algorithm of 500 to 1000 steps and followed by conjugate gradient algorithm from 500 to 1300 steps for estrogen receptor a and found to be stabilized at 1200 steps, for estrogen receptor p applied steepest descendent algorithm of 500 to 1000 steps and followed by conjugate gradient algorithm from 500 to 1200 and it is found to be stabilized at 1000 steps the minimization energies were tabulated in table 4.2. The GOLD fitness function has been optimized for a better prediction of ligand binding positions rather than ligand binding affinities (Jones *et ai,* 1997), and thus the best scoring solutions obtained in the various docking runs of receptor alpha and beta. Best scoring solutions are an indicator of best ligand binding positions rather than their relative strength of ligand binding.

Docking using a search algorithm:

The residues of the (Substrate binding region) estrogen receptor were used to define the putative ligand binding sites and the atoms corresponding to receptor in each case were written to the file 'cavity atoms'. The Genetic Algorithm settings in GOLD were set to 'Automatic' mode with an auto scaling of 1.0. By this method the program determines itself the optimum run parameters depending on the nature of the ligand and the receptor active site. Thus the parameters like the crossover frequency, number of genetic algorithm runs, mutation rates etc., are automatically adjusted by the program. The ligand atom-types were reset in GOLD and a flood-fill center radius of 20 A° was used to define the search space. The docking results were scored using the GOLD scoring function. In each case 50 Genetic Algorithm (GA) runs were performed.

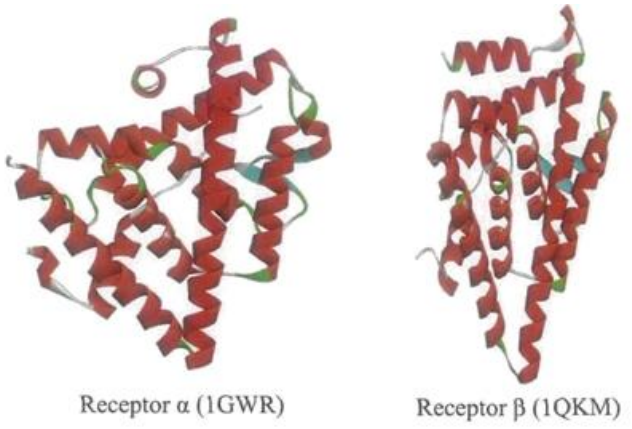

Figure 1: 3D structure of the receptors α and receptors β

The number of Hydrogen bonds and the number of non-bonded contacts between the ligand and the receptor were computed using SSViewer (Perugu *et al.,* 2013), DS Visualizer® (v.4.0, Accelrys Software Inc.) and LIGPLOT (Andrew *et at,* 1994) program.

3. Results and Discussion

Kinetic study of the oxidation of pentaammine cobalt

Isolation of bioactive compounds

The column packed with hexane extracted gum (containing three spots) is eluted with the solvents whose polarity gradually increased serially from 1- 4% of ethyl acetate: pet ether mixture. The first spot was obtained in fractions 100 to 120 at 2% ethyl acetate: pet ether with an yield of 0.5g, and second spot was eluted in the fractions 170 to 200 (3% ethyl acetate: pet ether) with an yield of 1.0g and finally third spot was eluted in the fractions 250 to 290 (4% ethyl

acetate: pet ether) with an yield of 1.5g and the results of which are depicted in **figure 1a-d.**

Characterization of bioactive compounds

The functional groups of the purified natural product were identified using standard FT-IR values. The reference values ~ 1697 and 1751 cm"[1] corresponds to carbonyl groups and peak around 1637 cm"[1] corresponds to alkene functionality. From this reference values, the purified compound is having keto, aldehyde and alkenic functional groups as illustrated in **figure 2a.** ^{1}H-NMR spectrum of a more purified sample has displayed chemical shifts in the aliphatic region (0.5-2 ppm), a deshielded resonance at 2.8 ppm, and an olefinic resonance between 5-6 ppm. There were no aromatic resonances in the spectrum as there is no peak in the aromatic region (7-8 ppm). This spectrum clearly showing that there is a presence of aldehyde functionality by a peak at 10 ppm as visualized in **figure 2b.**

The ^{13}C spectrum contained resonances primarily in the aliphatic region from 10-40 ppm, but the spectrum also contained resonances indicative of carbons attached to oxygen or otherwise deshielded by the electronegative environment (-80 ppm), olefinic carbons between 100-150 ppm, and two carbonyl carbons at 170 and 200 ppm. From the envisaged results we can infer that the purified compound is having one carbon attached to electronegative environment, two alkenic carbons and two carbonyl carbons as pictured in **figure 2c.** To confirm the structure of purified compound further we have carried out the 2D-NMR that is DEPT, COSY and NOESY. The ^{13}C NMR and DEPT135 spectra revealed that there are two carbonyl carbons in the compound that was analyzed. The proton singlet at 2.8 ppm that integrates to one proton in a deshielded signal that is consistent with a proton near to oxygen in the molecule. These data suggest that the *Saraca indica* compounds contains at least one ketone moiety. DEPT135 analysis confirmed the 50.1 ppm carbon to be a CH bearing carbon as shown in **figure 3a**. Together, the NMR data put forward that the *Saraca indica* compound contains two olefinic carbons, a terminal bond as well as one involving a quaternary center and a ketone moiety, and a carbon bearing a single proton likely located next to the ketone moiety.

Additional structural details of the *Saraca indica* metabolite was mined from the aliphatic region of the spectroscopic data. The ^{1}H NMR spectrum contains two singlet resonances at 1.1 ppm and 1.2 ppm that integrated to 3 H and 7 H respectively. These resonances suggested the presence of three methyl group on the compound. The COSY and DEPT135 spectra justified that the 1.1 ppm singlet was linked to a CH/CH_3 carbon at 24.6 ppm. The 7 H singlet at 1.2 ppm was found to be linked to two CH/CH_3 carbons at 22.5 and 32.8 ppm. Because these three methyl groups were all singlets and not split by any other proton signals .So, they all appeared to be attached to quaternary centers. Furthermore, the deshielded aliphatic carbon resonance at 32.8 ppm linked to a non-deshielded resonance at 1.2 ppm is characteristic. These resonances are consistent with Vicinal-dimethyl groups attached to a quaternary center.

The ^{1}H NMR spectrum also showed a number of signals with CH_2 splitting patterns between 1-2 ppm. Analyzing the DEPT and COSY spectrum, it revealed that in addition to the three methyl carbons on the major component of *Saraca indica* there were six additional carbons bearing hydrogens. All of these carbons (18.1, 23.0, 26.9, 29.6, and 40.5 ppm) showed multiple proton interaction suggesting these carbons had at least two attached protons. The DEPT135 confirmed that these six carbon signals were CH_2 signals for the *Saraca indica* compound. The rigid and sharp shapes of the CH_2 peaks suggested that they were involved in a ring system.

All the spectroscopical structural analysis of the *Saraca indica* compound obtained elucidated that the metabolite was likely to be a steroid as shown in **figure 3b.** The reason was based on the aliphatic nature of the compound, the non-aromatic ring system, the olefinic bonds, methyl groups as well as the carbonyl and hydroxyl/keto moieties that all are the common components of steroid compounds.Further analysis of the COSY data revealed that one olefinic proton at 6.8 ppm is not coupled with another olefinic proton suggesting this olefinic bond must contain a quaternary carbon as shown in **figure 3c**.

An olefinic bond containing a quaternary center is further supported by the presence of a quaternary carbon in the olefinic region of 100-150 ppm in the DEPT135 (Pretsch *et al.,* 2000). Identification of individual steroid components was made based on the mass spectral data obtained for our compound, and by comparison with literature information. The major components of the *Saraca Indica* (purified compound) retained the common Steroid core. Fragment peak at m/z 329 corresponding to the [M-l] as shown in **figure 3d.** High resolution mass spectroscopy spectrum revealing the molecular weight of the purified natural product is 328.0040 displayed in **figure 3e.** The isolated product was identified and its molecular formula is found to be $C_{21}H_{28}0_{3.}$

From the Ayurvedic, Unani context bark of *Saraca indica* was used to cure internal bleeding, hemorrhoids, uterine affections, breast cancer, menorrhagia, uterine fibroids, and Ieucorrhoea (Shilpakala *et al.,* 2009). The plant extracted ketosterol function is not known so far. However, ketosterol is found to be involved in the breast cancer/estrogen metabolism. The minimization scores of stable 3D-structures of four ligands viz., ketosterol (75.35381), estradiol (82.72538), Tamoxifen (34.37321) and raloxifene (20.96970) were tabulated in tablel. Minimized scores of two receptors viz., receptor a (-35114.53399) and receptor p (-19299.92042) were given in table 2. Prior docking the GOLD fitness scores for receptor-alpha (1GWR) and beta (1QKM) were given in Table 4.3. After performing the docking we found that the protein ligand interactions were in a good understanding with the receptor. The score for receptor-alpha is in the range of 85 to 100 and the score for

receptor-beta is in the range of 95 to 100. The ligands structures were displayed in the figure.4.3 were used for the docking.

3D structure of the ligands viz. ketosterol, estradiol, tamoxifen and raloxifene

We now report the relative binding affinities of ketosterol molecule atom 0-3, and 0-17 with ERa and ERp. This novel ketosterol analog to Estradiol can be used to ERa and ERp binding domains and could be potentially developed as novel drug. This ketosterol molecule also having the SERMs like activity. From the above observations, the binding of the ligand (Tamoxifen, raloxifene and estradiol) in the active site of receptor seem to be predominantly dependent on the non-bonded or hydrophobic interactions ligands (Tamoxifen, raloxifene and estradiol) makes with the estrogen receptor. The GOLD Fitness scores and the NB contacts have some correlation, indicating the importance of the non-bonded contacts in ligand binding. Hence, we attempted to compare the binding affinities of Tamoxifen, raloxifene and estradiol in receptor a and p after comparing and contrasting the binding positions receptor-a has more hydrogen bonds and less bond distance which represents good coordination with ketosterol. In receptor a ligand makes three Hydrogen bond interactions with HIS524, GLU353 and GLU353 of a-strand, out of which the shortest hydrogen bond distance is 2.47 A^0.

The other interactions are those of Van der Waals contacts with the receptor. In the case of receptor-beta the ligand makes two Hydrogen bond interactions with HIS475, ARG346 of a-strand, and the shortest hydrogen bond distance is 2.55 A^0. Plant extracted ketosterol (ligand) was showing the similar kind of activity like estradiol and SERMs and Plant extracted ketosterol may be expected with no side effects compare with SERMs. Ketosterol showed best binding affinity than default ligand which may acts as alternative to default ligand. Ketosterol was showed best hydrogen bond distance (2.47) than the default ligand (3.16) in receptor a and in the range of 95 to 100. The ligands structures were displayed in the figure.4.3 were used for the docking. Similarly, SERMs (Estradiol (2.39), Tamoxifen (3.11) and Raloxifene (2.98) and in case of Receptor p Estradiol (2.55), Tamoxifen (2.92) and Raloxifene (3.01)) later docking energies were noted control verses ketosterol shows best docking scoring solutions tabulated in Table 3.

Table. 4.1a: Energy minimization scores of ketosterol

Molecule Name	Minimization Algorithm	No of Steps	Energy	Initial Potential Energy (kcal/mol)	Potential Energy (kcal/mol)	Van der Waals Energy (kcal/mol)	Electrostatic Energy (kcal/mol)	Initial RMS Gradient (kcal/(mol x Angstrom)	Final RMS Gradient (kcal/(mol x Angstrom)
Ketosterol	Steepest Descent	500	174.02271	0.18908E+12	174.02771	10.64705	-11.42911	0.72217E+12	1.77998
		600	100.34728	0.18908E+12	100.34728	1.04745	-11.63123	0.72217E+12	2.20767
		700	93.50930	0.18908E+12	93.50930	0.82255	-11.25930	0.72217E+12	0.65316
		900	89.45829	0.18908E+12	89.45829	1.33792	-10.66649	0.72217E+12	0.30345
		1000	88.38150	0.18908E+12	88.38150	1.31924	-10.50929	0.72217E+12	1.25717
		1100	87.51855	0.18908E+12	87.18569	1.13855	-10.36989	0.72217E+12	1.03399
		1200	87.51855	0.18908E+12	87.18569	1.13855	-10.36989	0.72217E+12	1.03399
	Conjugate Gradient	900	75.35381	0.18908E+12	75.35381	-2.49033	-11.00399	0.72217E+12	0.09030
		1000	78.54884	0.18908E+12	75.35381	-2.49033	-11.00399	0.72217E+12	0.09100

Table. 4.1b: Energy minimization scores of estradiol

Molecule Name	Minimization Algorithm	No of Steps	Energy	Initial Potential Energy (kcal/mol)	Potential Energy (kcal/mol)	Vander Waals Energy (kcal/mol)	Electrostatic Energy (kcal/mol)	Initial RMS Gradient (kcal/(mol x Angstrom)	Final RMS Gradient (kcal/(mol x Angstrom)
Estradiol	Steepest Descent	1000	80.89551	53.01911	8.89551	-5.52362	-30.23642	23.64777	0.09895
		1100	81.89551	53.01911	8.89551	-5.52362	-30.23642	23.64777	0.09895
	Conjugate Gradient	1000	82.72538	53.01911	8.72538	-5.60588	-30.38660	23.64777	0.09648
		1200	82.72538	53.01911	8.72538	-5.60588	-30.38660	23.64777	0.09648

Table. 4.1c: Energy minimization scores of Tamoxifen

Molecule Name	Minimization Algorithm	No of Steps	Energy	Initial Potential Energy (kcal/mol)	Potential Energy (kcal/mol)	Vander Waals Energy (kcal/mol)	Electrostatic Energy (kcal/mol)	Initial RMS Gradient (kcal/(mol x Angstrom)	Final RMS Gradient (kcal/(mol x Angstrom)
Tamoxifen	Steepest Descent	1000	35.50454	433.29684	35.50454	3.30259	5.04922	274.81012	0.49442
		1100	35.22916	433.29684	35.22916	2.88589	5.12773	274.81012	0.09832
	Conjugate Gradient	1000	34.37321	433.29684	34.37321	1.82836	5.77323	274.81012	0.08021
		1500	34.37321	433.29684	34.37321	1.82836	5.77323	274.81012	0.08021

Table. 4.1d: Energy minimization scores of Raloxifene

Molecule Name	Minimization Algorithm	No of Steps	Energy	Initial Potential Energy (kcal/mol)	Potential Energy (kcal/mol)	Vander Waals Energy (kcal/mol)	Electrostatic Energy (kcal/mol)	Initial RMS Gradient (kcal/(mol x Angstrom)	Final RMS Gradient (kcal/(mol x Angstrom)
Raloxifene	Steepest Descent	1000	632.62571	116.240769	632.62571	38.67744	-10.27220	492.34050	0.56887
		1100	631.83161	116.240769	631.83161	37.72403	-10.40615	492.34050	0.09968
	Conjugate Gradient	1500	20.96970	116.240769	20.96970	36.04110	-18.76878	492.34050	0.09691

Table. 4.2a: Energy minimization scores of Estrogen receptor α

Molecule Name	Minimization Algorithm	No of Steps	Energy	Initial Potential Energy (kcal/mol)	Potential Energy (kcal/mol)	Vander Waals Energy (kcal/mol)	Electrostatic Energy (kcal/mol)	Initial RMS Gradient (kcal/mol x Angstrom)	Final RMS Gradient (kcal/mol x Angstrom)
1GWR .pdb	Steepest Descent	500	-26115.87854	1796290951.8	-26115.87854	-3410.93755	-27618.56040	351927871.5	2.64222
		1000	-28098.18551	1796290810.9	-28098.18551	-3305.93303	-29754.12580	351927871.5	0.80432
	Conjugate Gradient	1000	-35038.63960	1796290951.8	-35038.63960	-3710.92833	-35452.19756	351927871.5	0.14799
		1200	-35098.55849	1796290951.8	-35098.55849	-3707.52476	-35515.10380	351927871.5	0.11092
		1300	-35114.53399	1796290951.8	-35114.53399	-3706.23338	-35534.32678	351927871.5	0.14181
		1400	-35114.53399	1796290951.8	-35114.53399	-3706.23338	-35534.32678	351927871.5	0.14181

Table. 4.2b: Energy minimization scores of Estrogen receptor β

Molecule Name	Minimization Algorithm	No of Steps	Energy (Kcal/mol)	Initial Potential Energy (kcal/mol)	Potential Energy (kcal/mol)	Van der Waals Energy (kcal/mol)	Electrostatic Energy (kcal/mol)	Initial RMS Gradient (kcal/(mol x Angstrom)	Final RMS Gradient (kcal/(mol x Angstrom)
1QKM .pdb	Steepest Descent	500	-16187.67162	120025.05133	-16187.67162	-1822.98587	-16060.59735	25142.55864	2.29433
		1000	-17052.92042	120025.05133	-16187.67162	-1822.98587	-16060.59735	25142.55864	2.29433
	Conjugate Gradient	1000	-19299.27556	120025.05133	-19299.27556	-1750.79923	-19551.61026	25142.55864	0.09958
		1200	-19299.27556	120025.05133	-19299.27556	-1750.79923	-19551.61026	25142.55864	0.09958

Table. 4.3a: Estrogen receptor α and ligand interactions, binding scores and H-bonds

Ligand Name	α- Receptor's Name	Docking Energy (Kcal/mol)	No. of H-Bonds	Distance (Å)	Residues involved in H-Bond (Receptor)	Atoms of the Compound (Ligand).
Ketosterol	1GWR	-89.8905	3	3.14254 2.47974 2.9412	HIS524: ND1 GLU353:OE2 GLU353:OE1	O17 O3 O3
Estradiol		-88.2613	3	2.94612 2.39509 3.16773	HIS524: ND1 GLU353:OE2 GLU353:OE1	O17 O3 O3
Raloxifene		-108.864	1	3.07355 2.85536 3.17653 2.98447	SER456: O GLU385:OE1 CYS381:SG HIS547:ND1	O O O S
Tamoxifen		-89.6675	1	3.11157	SER527:OG	N

Table.4.3b: Estregen receptor β and ligand Interactions, binding scores and H-bonds

Ligand Name	β- Receptor's Name	Docking Energy (Kcal/mol)	No. of H-Bonds	Distance (Å)	Residues involved in H-Bond (Receptor)	Atoms of the Compound (Ligand).
Ketosterol	1QKM	-96.0048	2	2.60133 2.55601	HIS475:ND1 ARG346:NH2	O O
Estradiol		-97.9389	3	3.17281 3.19272 2.55859	LYS401:NZ LYS401:NZ HIS279:ND1	O O O
Raloxifene		-100.299	1	3.01714	GLU305:OE2	O
Tamoxifen		-95.5285	2	2.9296 3.12111	TYR397:OH LYS401:NZ	O O

Table.4.3c: Interactions of Estrogen receptor α and β with native ligand.

Inbuilt ligand bound	Distance (Å)	No. of H-Bonds	Residues involved in H-Bond (Receptor)	Atoms of the Compound (Ligand).
1GWR	2.94512	3	HIS524:ND1	O17
	3.16773		GLU353:OE1	O3
	2.39909		GLU353:OE2	O3
1QKM	2.60318	4	GLU305:OE1	O14
	3.02275		ARG346:NH2	O14
	2.71808		HIS475:ND1	O2
	3.08372		LEU476:N	O2

4. Conclusion

This *in-silico* study proves that plant extracted ketosterol inhibits the transcriptional activation function of estrogen receptor and Docking results have shown the better binding interactions compare with native ligand. Plant extracted ketosterol showed best binding affinity than default ligand which may acts as alternative to default ligand. Plant extracted ketosterol showed three hydrogen bonds with least distance than the default ligand and Estradiol, Tamoxifen and Raloxifene (SERMs) and ketosterol shows best docking energies.

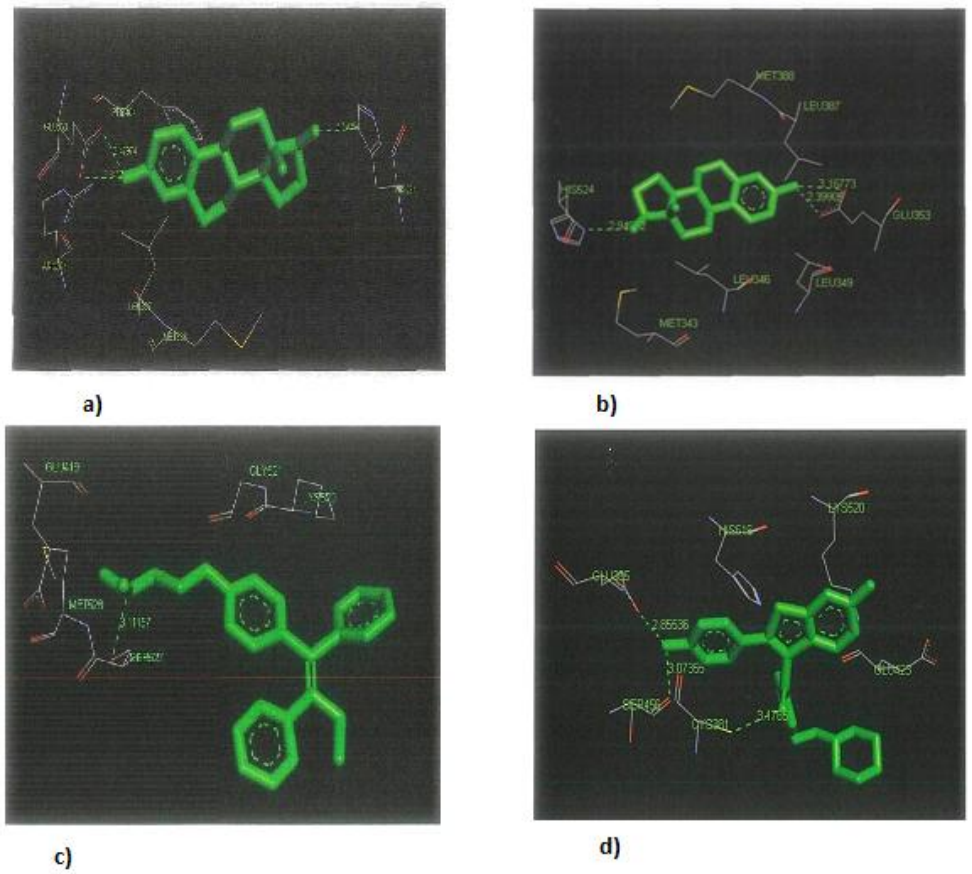

Figure 2: Interactions of the estrogen receptor alpha with ketosterol showing interaction with His524 (3.14 A°) and Glu353 (2.47 and 2.94 A^0) hydrogen bond distance respectively. **b:** Interactions of the estrogen receptor alpha with estradiol showing interaction with Glu353 (3.16 and 2.39A^0) and His524 (2.9A^0) hydrogen bond distance respectively. **C:** Interactions of the estrogen receptor alpha with estradiol showing interaction with Met528 (3.11 A^0) hydrogen bond distance. **d:** Interactions of the estrogen receptor alpha with estradiol showing interaction with Glu385 (2.85A^0), Cys381(3.17A^0) and Ser456 (3.07A^0) hydrogen bond distance respectively.

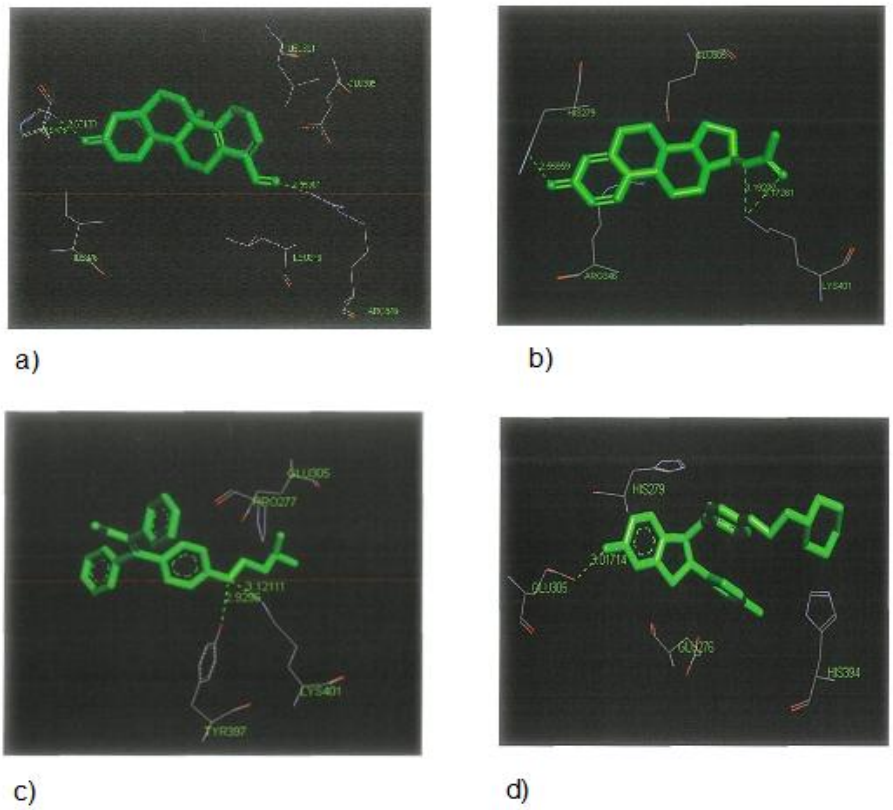

Figure 3: Interactions of the estrogen receptor a with ketosterol showing interaction with His475 (2.60A^0) and Arg346 (2.55A^0) hydrogen bond distance respectively. **b:** Interactions of the estrogen receptor a with estradiol showing interaction with His279 (2.55A^0), Lys401 (3.19 and 3.11A°) hydrogen bond distance respectively **c:** Interactions of the estrogen receptor a with Tamoxifen showing interaction with Tyr397 (2.92A^0) and Lys401 (3.12A^0) hydrogen bond distance respectively. **d:** Interactions of the estrogen receptor a with Raloxifene showing interaction with Glu305 (3.01 A^0) hydrogen bond distance respectively.

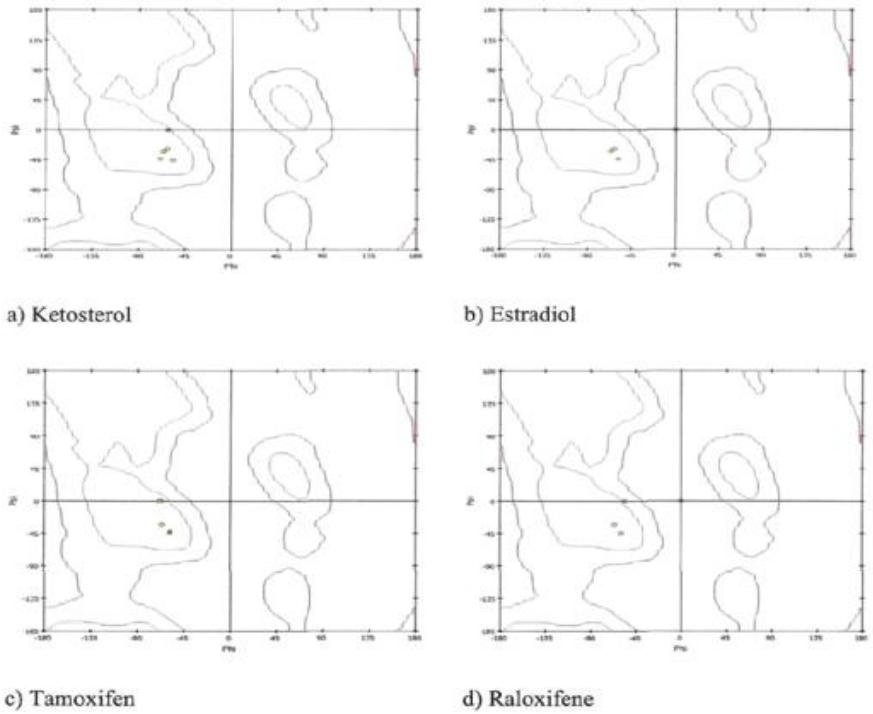

Figure 4: Ramachandran plot of the docked estrogen receptor p and ligand interactions of a). Ketosterol b). Estradiol c). Tamoxifen and d). Raloxifene were occupied in the left handed alpha helices only.

5. References

[1] COWANMM. Plant products as antimicrobialagents. Clin Microbiol Rev. **1999**, 12: 564-582.

[2] Chariandycm, Seaforthce, Phelpsrh, Pollardgv, Khambaybps. Screening of medicinal plants from Trinidad and Tobago for antimicrobial and in-secticidal properties. J Ethnopharmacol. **1999**, 3: 265-270.

[3] Bhavnanism, Ballowch. New agents for Gram-positive bacteria. Current Opin Microbiol. **2000**, 3: 528-534.

[4] Ahmadi, Mehmoodz, Mohammadf. Screening of some Indian medicinal plants for their antimicrobial properties. J Ethnopharmacol. **1998**, 62: 183-193

[5] Pradhan P., Joseph L., Gupta V. Saraca asoca (Ashoka): a review. Journal of Chemical and Pharmaceutical Research. **2009**, 1(1): 62–71.

[6] Bhalerao S. A., Verma D. R., Didwana V. S., Teli N. C. Saraca asoca (Roxb.), de. Wild: an overview. Annals of Plant Sciences. **2014**; 3(7): 770–775.

[7] Mittal A., Kadyan P., Gahlaut A., Dabur R. Nontargeted identification of the phenolic and other compounds of *Saraca asoca* by high performance liquid chromatography-positive electrospray ionization and quadrupole time-of-flight mass spectrometry. *ISRN Pharmaceutics*. **2013**, 10.

[8] Kumar S., Narwal S., Kumar D., Singh G., Arya R. Evaluation of antihyperglycemic and antioxidant activities of *Saraca asoca* (Roxb.) De Wild leaves in streptozotocin induced diabetic mice. *Asian Pacific Journal of Tropical Disease*. **2012**, 2(3): 170–176.

[9] Sasmal S., Majumdar S., Gupta M., Mukherjee A., Mukherjee P. K. Pharmacognostical, phytochemical and pharmacological evaluation for the antipyretic effect of the seeds of *Saraca asoca* Roxb. *Asian Pacific Journal of Tropical Biomedicine*. **2012**, 2(10): 782–786.

[10] Shirolkar A., Gahlaut A., Chhillar A. K., Dabur R. Quantitative analysis of catechins in *Saraca asoca* and correlation with antimicrobial activity. *Journal of Pharmaceutical Analysis*. **2013**, 3(6): 421–428.

[11] Sarojini N., Manjari S. A., Kanti C. C. Phytochemical screening and anthelmintic activity study of *Saraca indica* leaves extracts. *International Research Journal of Pharmacy*. **2011**, 2(5): 194–197.

[12] Middelkoop T. B., Labadie R. P. The action of *Saraca asoca* Roxb. de Wilde bark on the PGH2 synthetase enzyme complex of the sheep vesicular gland. *Zeitschrift fur Naturforschung. Section C: Biosciences*. **1985**, 40(7-8): 523–526.

[13] Suja M., Rajan S., Thiyagarajan Thirunalasundari B. J., Thenmozhi S. Pharmacognostical and phytochemical studies of an Ayurvedic drug *Saraca asoca*stem bark. *Journal of Pharmacy Research*. **2012**, 5(2): 1119–1121.

[14] Tusharkumar D. *Chemical investigation of phenolic constituents of two important medicinal plants Terminalia chebula and Saraca asoca* [Ph.D. thesis*]* Shri Jagdishprasad Jhabarmal Tibarewala University**; 2011**.

[15] Saha J., Mukherjee S., Gupta K., Gupta B. High-performance thin-layer chromatographic analysis of antioxidants present in different parts of *Saraca asoca* (Roxb.) de Wilde. *Journal of Pharmacy Research*. **2013**, 7(9):798–803.

[16] Maruthappan V., Shree K. S. Antiulcer activity of aqueous suspension of *Saraca indica* flower against gastric ulcers in albino rats. *Journal of Pharmacy Research*. **2010**, 3(1).

[17] Mathew N., Anitha M. G., Bala T. S. L., Sivakumar S. M., Narmadha R., Kalyanasundaram M. Larvicidal activity of *Saraca indica*, *Nyctanthes arbor-tristis*, and *Clitoria ternatea* extracts against three mosquito vector species. *Parasitology Research*. **2009**, 104(5): 1017–1025.

[18] Cibin T. R., Devi D. G., Abraham A. Chemoprevention of two-stage skin cancer *in vivo* by *Saraca asoca*. *Integrative Cancer Therapies*. **2012**, 11(3):279–286.

[19] Cibin T. R., Gayathri Devi D., Abraham A. Chemoprevention of skin cancer by the flavonoid fraction of *Saraca asoka*. *Phytotherapy Research*.**2010**, 24(5):666–672.