Chemical Constituents from Polyalthia Longifolia as Estrogen Receptor Modulators: An in Silico Molecular Docking Study

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***Abstract*—The estrogen receptor (ER) mediates most of the biological effects of estrogens at the level of gene regulation by interacting through its site-specific DNA and with other coregulatory proteins. The physiological effects of estrogen are manifested through ER's two isoforms, ERα and ERβ. These two isoforms (ERα and ERβ) display distinct regions of sequence homology. Human estrogen receptor alpha (ERα) is one of the most studied targets for in silico screening of bioactive compounds. It is a major target for treating different diseases, including the estrogen-dependent breast cancer. In this study we reported the molecular docking and ADMET descriptions of phytoconstituents from *P. longifolia* to get a deeper insight regarding its medicinal properties as anticancer agents. Structural insights into the antagonist–receptor interactions with human ERα (2IOG.pdb) was studied. Employing PyRx molecular docking tool the binding poses of phytoconstituents from *P. longifolia* were studied when compared with standard molecule (2IOG_cocrystal; 2-aryl indole derivative). The top scored four compounds with steroidal scaffold stigmasterol, Stigmasterol-3,5-diene, β-sitosterol and Longitriol exhibited higher binding energy values from -9.8 to -10.5 kcal/mol when compared with standard molecules 2IOG_cocrystal (2-aryl indole derivative) with -11.5 kcal/mol. The compounds exhibited strong hydrophobic and Van der Waal contacts with Leu525, Met528, Leu391, Met388, Leu346, Leu349, Leu387, Ala350, Phe404, and Ile424.**

The Pharmacokinetic, pharmacodynamics and toxicity profile of the designed inhibitors was evaluated employing SWISS ADME webserver. The properties like lipophilicity, water solubility, absorption, distribution, BBB permeability, CaCO2 permeability, human intestinal absorption, substrate and inhibition of CYP3A4 enzymes, lead likeness, and PIANS alerts were calculated for the top scored compound stigmasterol. Based on the current study, phytoconstituents with steroidal scaffold from *P. longifolia* could possibly be considered as a good starting point in further design of novel inhibitors against human Estrogen Receptor-α in the treatment of breast cancer, osteoporosis and uterine cancer.

I. INTRODUCTION

Estrogens are used in hormone replacement therapy (HRT) to prevent hot flashes, urogenital atrophy, and osteoporosis in postmenopausal women. HRT also may prevent heart disease, Alzheimer's disease, and colon cancer. Unfortunately, HRT has not lived up to its potential to improve the health of women, because estrogens have been associated with an increased incidence of breast and endometrial cancer. This relationship has hampered compliance with HRT severely and has sparked an intense pursuit for selective estrogen receptor modulators (SERMs) that have a safer profile [1-5].

The growing interest in using dietary natural plant estrogens (phytoestrogens), particularly those found in soy products, as a potential alternative to the estrogens in HRT. Fueled by observational studies showing a lower incidence of menopausal symptoms, osteoporosis, cardiovascular disease, and breast and endometrial cancers in Asian women who have a diet rich in soy products. Soy phytoestrogens prevent mammary tumors and bone loss in rodents and atherosclerosis of coronary arteries in monkeys. Soy protein relieves hot flashes in postmenopausal women

and attenuates bone loss in the lumbar spine of perimenopausal women. Furthermore, a high intake of dietary phytoestrogens is associated with a lower incidence of breast cancer in women [6-8].
The isoflavones, genistein, daidzein, and biochanin A, which are abundant in soybeans (34) and available widely as herbal tablets, are especially popular among postmenopausal women. Despite their popularity and putative health benefits it is clear to know the molecular mechanisms, safety, and efficacy of isoflavones before they can be recommended to postmenopausal women as an alternative to estrogens for Hormonal replacement therapy (HRT) [9].

Nuclear hormone receptors (NHRs) are transcription factors that play an important role in multiple diseases, such as cancer, inflammation, and metabolic disorders. The estrogen receptor (ER) is a member of the nuclear hormone receptor superfamily of ligand-activated transcription factors. Members of this class of proteins display a conserved structural organization consisting of a N-terminal domain (NTD), a highly conserved DNA binding domain (DBD), ligand-binding domain (LBD), Two activation function (AF) domains, AF1 and AF2, located within the NTD and LBD [10 (a) and (B)]. The ligand-binding domain (LBD) can adopt different conformations in response to substrate, agonist, and antagonist binding, leading to distinct transcription effects. Only accessible and most rigid portions, i.e., the ligand-binding domain (LBD) and the DNA-binding domain (DBD) were crystallized. Of these, the LBD is responsible for the binding of exogenous and endogenous molecules, for receptor homo- and hetero-dimerization and for the interaction with coregulatory proteins; it is characterized by a relevant flexibility and can adopt different conformations according to the bound ligand. The two isoforms of human ER (ERα and ERβ) encoded by two different genes have the physiological effects of estrogen are manifested through both ERα and ERβ. The ERα and ERβ receptor isoforms display distinct tissue distributions and signaling response [11].

The study of crystal structures of complexes with agonist and antagonist molecules identified the helix 12 (H12, residues 532–552) as the most important structural element of each LBD monomer, acting as a molecular switch between the active and inactive conformation of the receptor with “flip-flop” mechanism. After ligand interaction, the H12 helix undergoes a conformational change, assuming either an active conformation, where it is packed against helixes H3, H5/6, and H11 to close the binding site and create a surface essential for coactivators binding, or an inactive conformation, where it is displaced from the binding site and located in a groove formed by H3 and H5. As a consequence, the conformation of helix H12 can be used to assess the ability of a ligand to act as an agonist or antagonist in molecular dynamics simulations (Figure 1) [12].

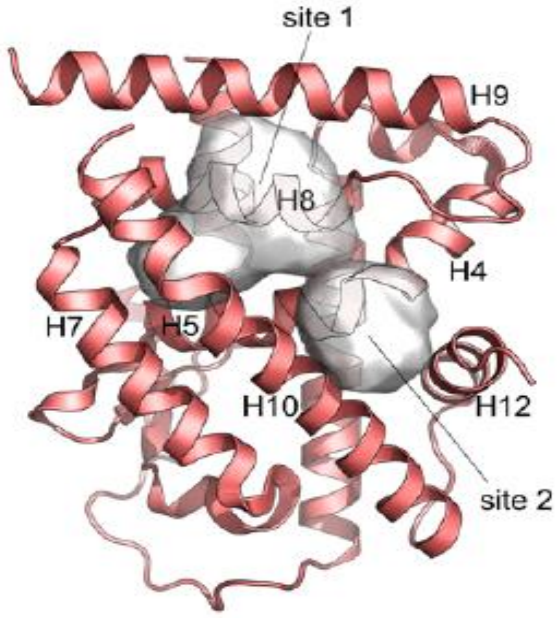

FIGURE 1: BOX PLOT SHOWING DYNAMIC CONFORMATION OF LBD FOR AGONIST AND ANTAGONIST POCKETS

ER antagonists and selective ER modulators (SERMs) have been shown to induce distinct conformations relative to the agonists, estradiol-17β (E2) and diethylstilbestrol, within the LBD of the receptor. SERMs, such as raloxifene and 4-hydroxytamoxifen (HT), which display mixed agonist/antagonist pharmacology, occupy the identical site as the agonists. The first crystal structure of an ERα LBD bound to its natural ligand 17β-estradiol (E2) showed that in a compact ellipsoid cavity, E2 is buried in a highly hydrophobic environment. Within this pocket (formed by 22 residues), hydroxyl groups in estradiol at positions 3 and 17 play a crucial role in orienting the steroid/ hormone ligand. These hydroxyl groups of the A and D rings are hydrogen bonded to Glu353 from H3, Arg394 from H5, and a water molecule and

His524 from H11 [13]. The crystal structures of the human ERβ bound to genistein, estradiol, and rat ERβ to raloxifene assert the importance of hydrogen bond network on the opposite sides of the respective ligands [14].

Recently, raloxifene as SERM has been approved for the prevention and treatment of osteoporosis. Despite of its desirable effects its low potency than estrogens at increasing bone mineral density also increases hot flashes and no improvement in cognitive function created a quest for superior SERMs for HRT continues [15]. From literature it was established that soya isoflavone, genistein has agonist activity for both ERα and ERβ, but that genistein's affinity for ERβ is considerably greater; genistein's affinities for Erβ and ERα were determined to be 8.4 nM and 145 nM, respectively. For daidzein, the corresponding values were 100 nM and 420 nM, indicative of its much lower affinity for these receptors. At saturating concentrations, both genistein and daidzein could interact with either of these receptors to activate transcription from estrogen response elements, at least as effectively as the physiological ligand 17β-estradiol [16].

Plant and plant products are being used as a source of medicine since long. According to the World Health Organization, more than 80% of the world's population, mostly in poor and less developed countries depend on traditional plant-based medicines for their primary health care needs [17]. Polyalthia longifolia cv. pendula (Annonaceae) is native to the drier regions of India and is locally known as "Ashoka" and is commonly cultivated in India, Pakistan, and Sri Lanka. P.longifolia, although an ornamental tree, finds its reference in Indian medicinal literature owing to its popular Hindi name Ashoka. This plant is used as an antipyretic agent in indigenous systems of medicine [18]. Pharmacologic studies on the bark and leaves of this plant display effective antimicrobial activity, [19] cytotoxic function, [20] and hypotensive effects. The genus Polyalthia belongs to the family Annonaceae. The Polyalthia genus is considered to be of medicinal importance because of the presence of clerodane diterpenoids and alkaloids in various parts of the plant. India has 14 species of Polyalthia.

P. Longifolia is a tall handsome evergreen plant belonging to Annonaceae family cultivated throughout India and usually planted as an ornamental street tree as it alleviates noise pollution. *P. Longifolia* is normally called as false Ashoka, Buddha Tree, Green champa, Indian mast tree, and Indian Fire tree. It shows symmetrical pyramidal growth along with willowy weeping pendulous branches and long, alternate, stipulate, mildly aromatic, shinning, glabrous, narrowly lanceolate leaves and margin is markedly undulate. The tree is usually growing over 30 ft in height. Fruits are borne in clusters of 10-20, typically ovoid in shape. At the beginning fruits are green in colour however turns purple or black when ripe. Seeds are ovoid and pale brown in colour with a longitudinal groove. Nearly all parts of PL plant have their applicability in traditional system of medicine to treat fever, skin diseases diabetes hypertension and helminthiasis including vitiated condition of vatta and pitta. The plant extract and isolated compounds were investigated for variety of biological activities such as antibacterial activity, cytotoxicity and antifungal activity [21]. *P. longifolia* mainly contains diterpenoids, alkaloids tannins, and mucilage (Figure 5). The chief components of the plant are O-methyl bulbocapnine-N-oxide, polyfothine, N-methylnandigerine-N-oxide, oliveroline-N-oxide, pendulamine A, N-pendulamine B, 8-oxopolyalthiane, 16-oxo-5, 13-halimadien-15-oic acid, 16-Oxo-3, 13-clerodadien-15-oic acid, 16-hydroxycleroda-3, 13-dien-16, 15-olide. Two clerodane-type diterpenoids, 16α-hydroxycleroda- 3,13(14)Z-dien-15,16-olide and 16-oxo-cleroda-3,13(14)E-dien-15-oic acid. A new γ-methoxybutenolide clerodane diterpene 2 has been isolated from the petroleum ether extract of the bark of *P. longifolia*.

Computational techniques have often been used to complement experimental studies in order to assist with data analysis as well as improve results. In this instance, rapid in silico screening can be used not only to help identify and prioritize which class of compounds to screen, but also reduce the number of compounds to be tested. Docking is one of the popular techniques commonly used for a number of purposes, e.g., ligand pose prediction, ligand binding affinity prediction as well as identifying potential actives from a library of decoys in virtual screening. The present study aims to identify the binding pose and affinity of chemical constituents from *P. longifolia* as estrogen receptor modulators targeting Estrogen receptor α (ER α) through in silico molecular docking approach.

II. MATERIALS AND METHODS

PROTEIN AND LIGAND PREPARATION

Based on the literature survey the 3D structures of different chemical constituents (diterpenoids, alkaloids, steroids tannins etc.,) were downloaded from IMPPAT database (https://cb.imsc.res.in/imppat/phytochemical/Polyalthia%20longifolia) in SDF file format. The ligands were further prepared using PyRx tool, they were minimized using the energy minimization module comprised with universal force field (UFF). The minimized ligands were converted into. pdbqt format using Auto Dock ligand format tool. The 3D crystal structure of human Estrogen Receptor-α complexed with 2-Aryl indole derivative (2IOG.pdb) was downloaded from protein data bank and further protein preparation was done using Swiss PDB viewer and AutoDock 1.5.6 ADT tools. Polar hydrogens were added with deleting water molecules and using CHARMM force field the protein was energy minimized. The prepared protein was validated by using the Ramachandran plot (Figure 2).

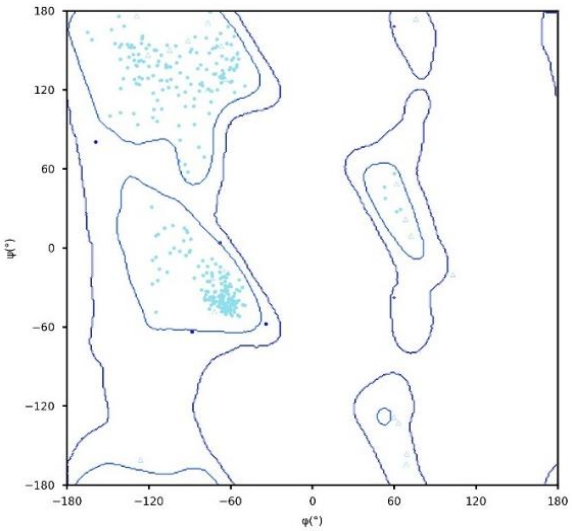

FIGURE 2: RAMACHANDRAN PLOT FOR THE PREPARED PROTEIN 2IOG.PDB GENERATED USING RAMPLOT WEBSERVER. HERE, CYAN, BLUE AND RED (DOTS/TRIANGLES) REPRESENT TORSION ANGLES OF FAVOURED, ALLOWED AND DISALLOWED REGIONS RESPECTIVELY; DOT REPRESENTS RESIDUES OTHER THAN GLYCINE AND TRIANGLES REPRESENTS GLYCINE

IDENTIFICATION OF ACTIVE SITES AND GRID GENERATION

Using Protein-ligand interaction profile (PLIP) tool, https://plip-tool.biotec.tu-dresden.de/plip web/plip/index, the amino acids surrounding the active site was identified and the amino acid residues were noted to generate the grid box in the protein's active site with center of a box dimension x= 30.034, y= 0.0323 and z= 24.624 Å and box size of x= 30.4330, y= 25.0 and z= 29.593 Å.

MOLECULAR DOCKING

Keeping whole protein as rigid except binding pocket residues as flexible, the energy minimized ligands were docked into the catalytic pocket of human Estrogen Receptor-α (2IOG.pdb). A configuration text file was generated to keep 9 binding poses based on RMSD and energy range was kept at 4 with exhaustive ness at 9. The docked poses were visualized using Receptor-Ligand interactions module of BIOVIA/Discovery Studio.

ADMET CALCULATION:

The drug likeness properties, pharmacokinetic and physicochemical descriptors of small molecules were predicted using SWISS ADME webserver.

III. RESULTS AND DISCUSSION

PROTEIN AND LIGAND PREPARATION

Based on the reported literature the 3D structures of chemical constituents from *P. longifolia* were collected from Indian Medicinal Plants, Phytochemistry and Therapeutics (IMPPAT) database. The 3D structures were browsed by choosing the Indian medicinal plant name from a drop-down menu in the IMPPAT website [22]. Few chemical constituents from *P. longifolia* were represented in the Figure 3. The molecules were further energy minimized employing Open Babel (OB) module of PyRX tool. OB is a chemical toolkit that is used in conjunction with the PyRx virtual screening software. PyRx leverages OB to handle tasks like converting chemical file formats, preparing ligands for docking, and performing energy minimization. PyRx utilizes OB to convert ligands to AutoDock Ligand format (PDBQT) for docking purposes. The 3D protein structure of human estrogen receptor alpha ligand-binding domain in complex with

inhibitor compound 2-aryl indoles (2IOG.pdb) was downloaded from RCSB Protein Data Bank (RCSB PDB). [https://www.rcsb.org/].

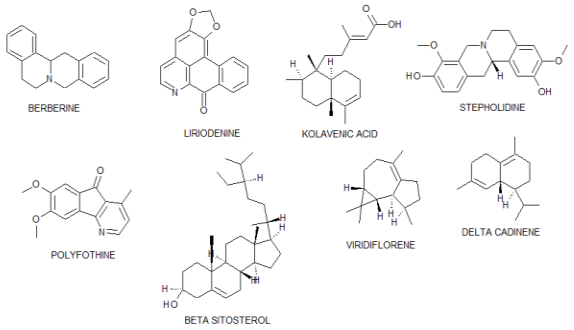

FIGURE 3: STRUCTURES OF VARIOUS PHYTOCHEMICAL CONSTITUENTS FROM *P. LONGIFOLIA*

MOLECULAR DOCKING

The obtained 3D protein structure of protein was first prepared by employing Swiss-Pdb Viewer [https://spdbv.unil.ch/] module. A grid box was generated in the protein's active site with center of a box dimension x= 30.034, y= 0.0323 and z= 24.624 Å and box size of x= 30.4330, y= 25.0 and z= 29.593 Å. Finally, the energy minimized ligands were docked into the catalytic pocket of human Estrogen Receptor-α (2IOG.pdb). Vina Wizard of PyRX tool was employed to generate the top docked poses with lowest RMSD to the initial pose. The binding energy values along with number of hydrogen bonds and interacting amino acids of the top 10 ligands was represented in the Table 1.

TABLE 1: BINDING AFFINITY (KCAL/MOL) VALUES PREDICTED FOR THE TOP SCORED PHYTOCHEMICAL CONSTITUENT'S FROM *P. LONGIFOLIA* AGAINST HUMAN ESTROGEN RECEPTOR-A (2IOG.PDB).

Compound name	Binding affinity (kcal/mol)	Interacting amino acids
Stigmasterol	-10.5	Leu525, Met528, Leu391, Met388, Leu346, Leu349, Leu387, Ala350, Phe404, Ile424.
Stigmasterol-3,5-diene	-10.2	Leu525, Leu391, Met388, Leu346, Leu349, Leu387, Ala350, Phe404
β-sitosterol	-10.2	Met388, Leu346, Phe404, Leu349, Ala350, Leu387
Longitriol	-9.8	--
16-α-methoxy-cleroda-3,13z-dien-16,15-olide	-9.8	Leu346, Leu525,
16-hydroxy-halima-5 (10),13-dien-16,15-olide	-9.6	Met421, Leu346
16-α-hydroxy-cleroda-3,13(14)z-dien-15,16-olide	-9.6	Leu346, Ile424, Met421
Stepholidine	-9.6	Leu346, Ile424, Met421
Kolavenic acid	-9.6	Leu346, Ile424, Met421, Ala350, Leu387
Estradiol	-10.3	Glu353, Ala350, Leu391, Leu387, Met288, Ile424
2IOG_cocrystal	-11.8	Leu391, Leu387, Ala350, His524, Met421, Cys530, Trp383, Leu536, Lys531

From the molecular docking results out of 240 chemical constituents from *P. longifolia* the top scored hits were stigmasterol, Stigmasterol-3,5-diene, β-sitosterol and Longitriol, with binding affinity values of -10.5, -10.2, -10.2 and -9.8 kcal/mol (Figures 4 and 5). These top scored compounds have steroidal ring in their structures and formed hydrophobic and Van der Waal interactions with the active site amino acids Leu525, Met528, Leu391, Met388, Leu346, Leu349, Leu387, Ala350, Phe404, and Ile424. The standard molecules 2IOG_cocrystal (2-aryl indole derivative) and estrogen receptor modulator (estradiol) showed higher binding affinity values of -11.8 and -10.3 kcal/mol respectively by forming hydrogen bonding interaction with Glu353 and hydrophobic interactions with Leu391, Leu387, Ala350, His524, Met421,

Cys530, Trp383, Leu536, and Lys531. As it was observed from the Figure 5, all the docked compounds exhibited non polar interactions due to the hydrophobic pocket at the active site and also throughout the protein structure.

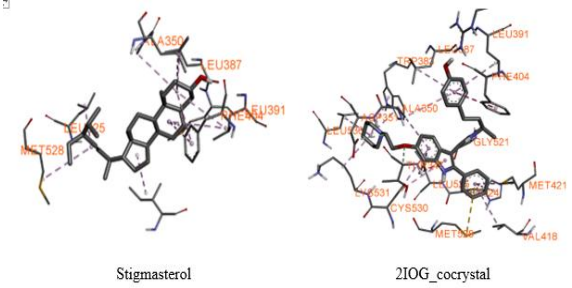

FIGURE 4: 3D INTERACTION DIAGRAM OF STIGMASTEROL (-10.5 KCAL/MOL) AND 2IOG_COCRYSTAL (-11.8 KCAL/MOL) IN THE ACTIVE SITE OF 2IOG.PDB

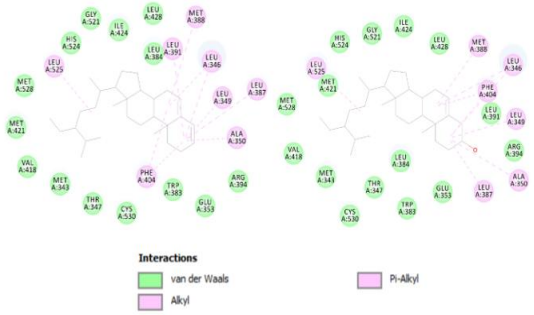

FIGURE 5: 2D INTERACTION DIAGRAM OF STIGMASTEROL-3,5-DIENE (-10.2 KCAL/MOL) AND B-SITOSTEROL (-10.2 KCAL/MOL) IN THE ACTIVE SITE OF 2IOG.PDB

IV. ADMET PREDICTION

ADMET (Absorption, Distribution, Metabolism, Excretion and Toxicity) prediction is a crucial step in drug discovery, helping to identify potential issues with drug candidates early on. It involves using computational models to predict how a drug will behave in the body, which can impact its efficacy and safety. These predictions are often used to guide lead optimization, helping chemists identify and correct potential ADMET liabilities in a drug series. The molecular structure of the stigmasterol was introduced in simplified molecular-input line-entry specification (SMILES) nomenclature into the ADME/Tox web tools SwissADME and pkCSM-pharmacokinetics. The detailed ADME/Tox properties provided from the web tools to represent the ADME/Tox profile.

To this end, the Brain or IntestinaL Estimated permeation method (BOILED-Egg) is proposed as an accurate predictive model that works by computing the lipophilicity and polarity of small molecules. Lipophilicity (WLOGP) on Y axis and and polarity (tPSA) computation on X-axis (Figure 6).

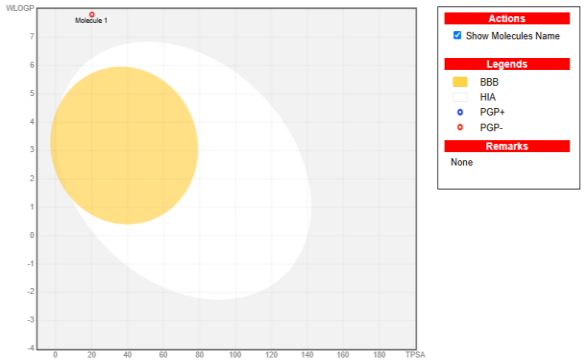

FIGURE 6: BOILED-EGG PLOT GENERATED BY SWISS-ADME WEB SERVER FOR THE STIGMASTEROL

The Bioavailability Radar from SwissADME (Figure 7) is a tool used to assess the drug-likeness of molecules based on six physicochemical properties: lipophilicity (LIPO), size (SIZE), polarity (POLAR), solubility (INSOLU), instauration (INSATU), and flexibility (FLEX). These properties are considered important for a compound to be well-absorbed and distributed in the body, leading to good bioavailability.

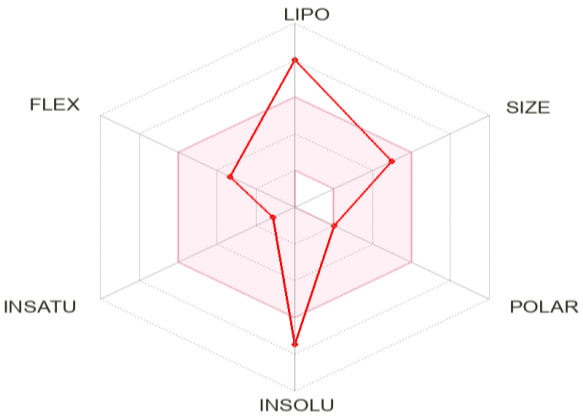

FIGURE 7: BIOAVAILABILITY RADAR GRAPH GENERATED BY SWISS-ADME WEB SERVER FOR THE STIGMASTEROL

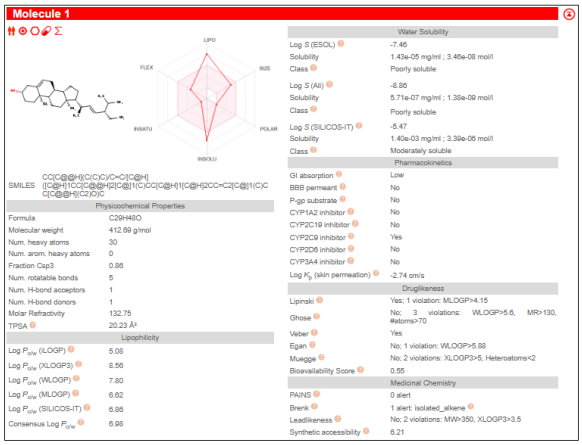

FIGURE 8: PHYSICOCHEMICAL PROPERTIES AND ADMET PROPERTIES GENERATED BY SWISS-ADME WEB SERVER FOR THE STIGMASTEROL

Absorption was predicted from water solubility, lipophilicity and percentage of intestinal human absorption (HIA) properties (Figure 8). Water solubility was predicted using the Silicos IT Log Sw descriptor of SwissADME. Log Sw values for our compound stigmasterol was predicted to be −5.47. In the SwissADME Log Sw scale, compounds with values less than (more negative than) −6 are considered to be poorly soluble. Lipophilicity was assessed using the logarithm of the n-octanol/water partition coefficient, which was predicted using the Consensus LogP o/w descriptor of SwissADME. Log Po/w is closely related to transport processes, including membrane permeability, and distribution to different tissues and organs. A general guide for good oral bioavailability (good permeability and solubility) is to have a moderate logP ($0 < \log P < 3$). For our compound stigmasterol, the predicted values of logP o/w were 6.98. The logSw and logPo/w predictions indicated a correlation between solubility and lipophilicity. The values of TPSA in $Å^2$ within the range $140 > TPSA < 60$ are indications of excellent intestinal absorption and good blood-brain barrier penetration, respectively. The TPSA for stigamsterol was found to be 20.23 $Å^2$ indicating poor intestinal absorption.

V. CONCLUSION

Many molecules are being studied for their therapeutic potential. A lot of them have origin in natural products, with notorious relevance for drug discovery. Bioinformatics has become an important tool in the screening of these molecules before the in vitro and in vivo analysis. In this study phytoconstituents from *P. longifolia* were evaluated for their estrogen receptor modulatory activity against human Estrogen Receptor-α (2IOG.pdb).

The 3D structures of phytoconstituents were downloaded from IMPPAT database and were further energy minimized employing Open Babel module of PyRX tool. The energy minimized ligands were docked into the catalytic pocket of human Estrogen Receptor-α (2IOG.pdb). The top scored four compounds with steroidal scaffold stigmasterol, Stigmasterol-3,5-diene, β-sitosterol and Longitriol exhibited higher binding energy values from -9.8 to -10.5 kcal/mol when compared with standard molecules 2IOG_cocrystal (2-aryl indole derivative) and estrogen receptor modulator (estradiol) with -11.8 and -10.3 kcal/mol respectively. The compounds exhibited strong hydrophobic and Van der Waal contacts with Leu525, Met528, Leu391, Met388, Leu346, Leu349, Leu387, Ala350, Phe404, and Ile424.

The Pharmacokinetic, pharmacodynamics and toxicity profile of the designed inhibitors was evaluated employing SWISS ADME webserver. The properties like log p, log s, BBB permeability, $CaCO_2$ permeability, human intestinal absorption, substrate and inhibition of CYP3A4 enzymes, and PIANS alerts were calculated. From ADMET studies the selected top ranked compound showed poor log p values and plasma protein binding. Based on the current study, phytoconstituents with steroidal scaffold from *P. longifolia* could possibly be considered as a good starting point in further design of novel inhibitors against human Estrogen Receptor-α in the treatment of breast cancer, osteoporosis and uterine cancer.

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