A Computational Drug Designing From Active Product of Herbal Plant Ochna Squarrosa to Relieve Menstrual Complexities

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Abstract

Ochna squarrosa (Golden Champak), a Bangladeshi herbal plant known locally as Sheuri, has for long been used for treating menstrual complexities. Although root decoction of the related species variants has been reported to contain active compound Ochnaflavone- a derivative of isoflavone- this chemical's presence in *O. squarrosa* was far from confirmed. Furhtermore, the molecular mechanism of action of the chemical is yet to be identified. Here, we report the presence of Ochnaflavone in the plant. Moreover, our computational study reveals a plausible target protein where the active compound binds. This study confirms the basis of the traditional herbal practice and can be useful for further developing a synthetic drug. This in return, we hope, will shift the current use of Ochnaflavone as 'observational medicine' to 'evidence based medicine'.

Keywords: Ochna Squarrosa, Estrogen Receptor, Isoflavone, Molecular Docking.

1 INTRODUCTION

Estrogen receptors bind hormone estrogen, alternatively known as 17β-estradiol. There are two distinct forms of the estrogen receptor- they are referred to as **α** and **β**. Each of these forms is encoded by a separate gene (*ESR1* and *ESR2*, respectively). Hormone-activated estrogen receptors form dimers and since the two forms are coexpressed in many cell types, the receptors may form ERα (αα) or ERβ (ββ) homodimers or ERαβ (αβ) heterodimers (Li X *et al.* 2004) [1] Estrogen receptor alpha and beta show significant overall sequence homology and both are composed of five domains.

The N-terminal domain can activate gene transcription in the absence of bound ligand (e.g., the estrogen hormone). While this region is able to activate gene transcription without ligand, this activation is weak and more selective compared to the activation provided by the E domain. The C domain, which is actually the DNA-binding domain, binds to estrogen response elements in DNA. The D domain works as a hinge region that connects the C and E domains. The E domain contains the ligand binding cavity as well as binding sites for coactivator and corepressor proteins. The E-domain in the presence of bound ligand is able to activate gene transcription. The C-terminal F domain function is not entirely clear and is variable in length (Ascenzi P *et al.* 2000, Bourguet W *et al.* 2006) [2][3].

The ER's helix 12 domain plays a crucial role in determining interactions with coactivators and corepressors and, therefore, the respective agonist or antagonist effect of the ligand (Ascenzi P 2000, Bourguet W 2006) [2][3].

Isoflavones comprise a class of organic compounds, often naturally occurring, related to the isoflavonoids (Kaufman PB *et al.* 1997, Heber D *et al.* 2008) [4][5]. Many act as phytoestrogens in

mammals. Being phytochemicals, they are able to be termed antioxidants because of their ability to trap singlet oxygen. Some isoflavones, in particular soy isoflavones, when studied in populations eating soy protein, have indicated that there is a lower incidence of breast cancer and other common cancers because of its role in influencing sex hormone metabolism and biological activity through intracellular enzymes, protein synthesis, growth factor actions, malignant cell proliferations, differentation and angiogenesis (Heber D *et al.* 2008) [5]. Isoflavones are produced almost exclusively by the members of the *Fabaceae* (that is *Leguminosae*, or bean) family.

FIGURE 1: *Ochna squarrosa* plant. The plant is found abundantly in Chittagong Hill Tracts (CHT) and in the northern zone of Mymensingh district in Bangladesh. Although plants from both the location seems to be similar, slight variations in phenotype may well be subject to further investigation. In Bangladesh varieties of the plant, if any, is not well classified.

Ochna squarrosa (**FIGURE 1**) is small sub-deciduous tree. The tree is popularly known as Sheuri in local language. Leaves of the plant are alternate, 6-12 cm long, obovate, elliptic, sharply serrate. Flower of the plant is fragrant and it shoots from the ends of short, lateral branches or scars of fallen leaves in corymbose racemes. Plant contains fruit of 3-6 drupes, 6 mm long, oblong-ovoid, black, surrounded by the persistent calyx. The plant is found in the forests of Chittagong, Chittagong Hill Tracts, Cox's Bazar, Sylhet, Mymensingh.

PatchDock (http://bioinfo3d.cs.tau.ac.il/PatchDock/) is a computational tool for determining protein ligand or protein protein interaction. Its algorithm is based on object recognition and image segmentation techniques used in Computer Vision. It actually mimics human vision and extracts image data from protein structure files such as in .pdb file extension. Docking can be compared to assembling a jigsaw puzzle. When solving the puzzle it is tried to match two pieces by picking one piece and searching for the complementary one (Mashiach E *et al.* 2010) [6]. A puzzle-solver concentrates on the patterns that are unique for the puzzle element and look for the matching patterns in the rest of the pieces. PatchDock employs a similar technique. Given two molecules, their surfaces are divided into patches according to the surface shape. These patches correspond to patterns that visually distinguish between puzzle pieces. Once the patches are identified, they can be superimposed using shape matching algorithms (Schneidman-Duhovny D *et al.* 2003, Duhovny D NR *et al*. 2002) [7][14]. The algorithm has three major stages:

- Molecular Shape Representation - in this step PatchDock compute the molecular surface of the molecule. Next, it applies a segmentation algorithm for detection of geometric patches (concave, convex and flat surface pieces). The patches are filtered, so that only patches with 'hot spot' residues are retained.

- Surface Patch Matching - PatchDock applies a hybrid of the Geometric Hashing and Pose-Clustering matching techniques to match the patches detected in the previous step. Concave patches are matched with convex and flat patches with any type of patches.
- Filtering and Scoring - the candidate complexes from the previous step are examined. It discards all complexes with unacceptable penetrations of the atoms of the receptor to the atoms of the ligand. Finally, the remaining candidates are ranked according to a geometric shape complementarity score.

Pocket-Finder (http://www.modelling.leeds.ac.uk/pocketfinder/), an online computational tool to predict active site within a structure, is based on the Ligsite algorithm written by Hendlich *et al.* (1999) [8]. Pocket-Finder was written to compare pocket detection with our new ligand binding site detction algorithm Q-SiteFinder**.**

2. METHOD

2.1 Preparation of Root Decoction

Ochna squarrosa root decoction was collected from local herbal vendors. The root preparation was prepared using the standard isolation procedure. A very traditional approach was followed to prepare the root decoction. Local *Kabiraj* and *Homeopathic* physicians have been following the same procedure for ages. At first, the dry herbal ingredients was put in an earthenware or stainless steel pot and cold water was added until the water level is 3–4 cm above the herbs. The herbs were left to soak in the water for at least 1 hour. A pot on the cooker was placed and strong heat was applied to bring it to the boil. Once the liquid is boiling, heat was turned down and the herbs were simmered for 20 minutes, and then the decoction was strained from the pot into a receptacle. Another 200 ml of cold water was poured onto the herbs in the pot and cook for a further 20 minutes. The resulting liquid was strained into the receptacle holding the first decoction, thus mixing the two decoctions together. The total liquid obtained was about 200–250 ml. Finally, the decoction was divided into two or three doses and was taken these over the course of the day, warming each dose before drinking it.

2.2 Proof of Presence of Isoflavone

It is widespread in literature that isoflavone is responsible for relieving the menstrual complexity (Kennelly EJ *et al.* 2002) [9]. Presence of Isoflavone in species phylogenetically and phenotypically similar plants prompted us to isolate this active compound from the root decoction. After the root extract was purified, the presence of isoflavone as an active compound in the extract was predicted using chemical confirmation tests.

2.3 Computational Prediction

The 3D isoflavone structure was generated and was subsequently docked to protein 3D structure of Estrogen receptor. Protein Data Bank (PDB) (http://www.pdb.org/) file of Oyster estrogen receptor (PDB id: 3LTX) was collected from PDB database. The estrogen binding residues were conserved among Human and Oyster estrogen receptor- both the enzyme contains Asn and Gln in its active site. Estrogen receptor showed significantly positive results for receptor-ligand type interaction.

3. RESULTS

3.1 Proof of Presence of Isoflavone

The extract was purified using column chromatography and filtration. Purity of the product was fairly good for carrying out the chemical tests carried out later on. Isoflavone gave positive tests for its characteristic presumptive tests (S. F. Dyke *et al.* 1961) [10].

Flavone and isoflavone are structurally similar. So, presumptive tests for Isoflavone were carried out for portion common to both the structure. When boiled with concentrated potassium hydroxide solution, flavones (I) gave a mixture of four products- salicylic acid (III), acetophenone (IV), o-

hydroxyacetophenone (V) and benzoic acid (VI) (**FIGURE 2**). These products arise in pair- III and IV, and V and VI together (J.B. Harborne 1965, J.B. Harborne 1967) [11][12][19]. Both of them has a common precursor, a diketone, when the flavones pyrone ring opens to produce o-hydroxydibenzoylmethane (II). The products (III to VI) were individually identified by their characteristic chemical reactions.

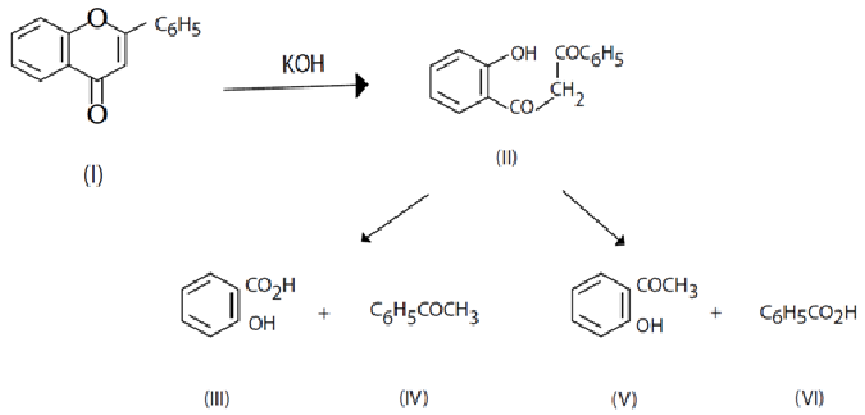

FIGURE 2: Confirming Flavone in root decoction. Purified root decoction conataining flavones was treated with potassium hydroxide which splitted the compound in two possible way. (See text for detail).

Isoflavone gives similar reaction. Fusion with potassium hydroxide breaks down the molecule in two fragments- one of them is resorcinol (**FIGURE 3**). An additional step to hydrolyze it with ethanolic potassium hydroxide permits the intermediates.

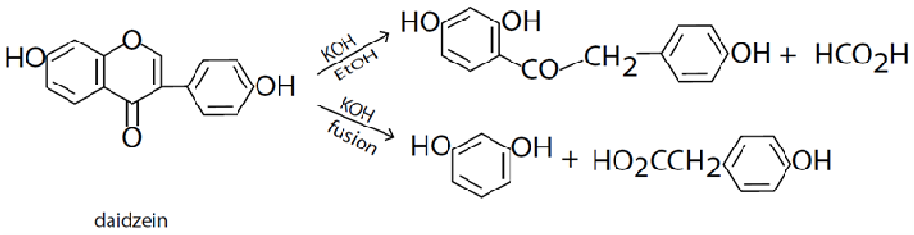

FIGURE 3: Confirming Isoflavone in root decoction. Didazein has similar structure to isoflavone. Isoflavone gives similar reaction to that of flavone's one (See text for detail).

3.2 Computational Prediction

Computational drug designing has become one of the most effective ways to screen a ligand's ability as a drug. In biological terms, ligands are small molecules that interact with a protein. Isoflavone is a small molecule that mimics the structure of estrogen. Estrogen, a steroid hormone, is helps to alleviate menstrual cramps and other complexities. Estrogen binds to Estrogen receptor intracellularlyand relay a signal by binding straight to DNA response elements (Paul S. Cooke *et al.* 1998) [13]. Isoflavone shows remarkable similarity to the bound state intermediate of the estrogen-estrogen receptor complex. When we computationally docked isoflavone to estrogen receptor (Figure-4), the binding affinity was very high and it bound at the same site where estrogen binds indicating the fact that it can work as a synthetic activator for the receptor (**FIGURE 4**). Isoflavone, as an estrogen agonist, can even induce estrogen action in the absence of its usual ligand, that is estrogen, and thereby relieve menstrual pain.

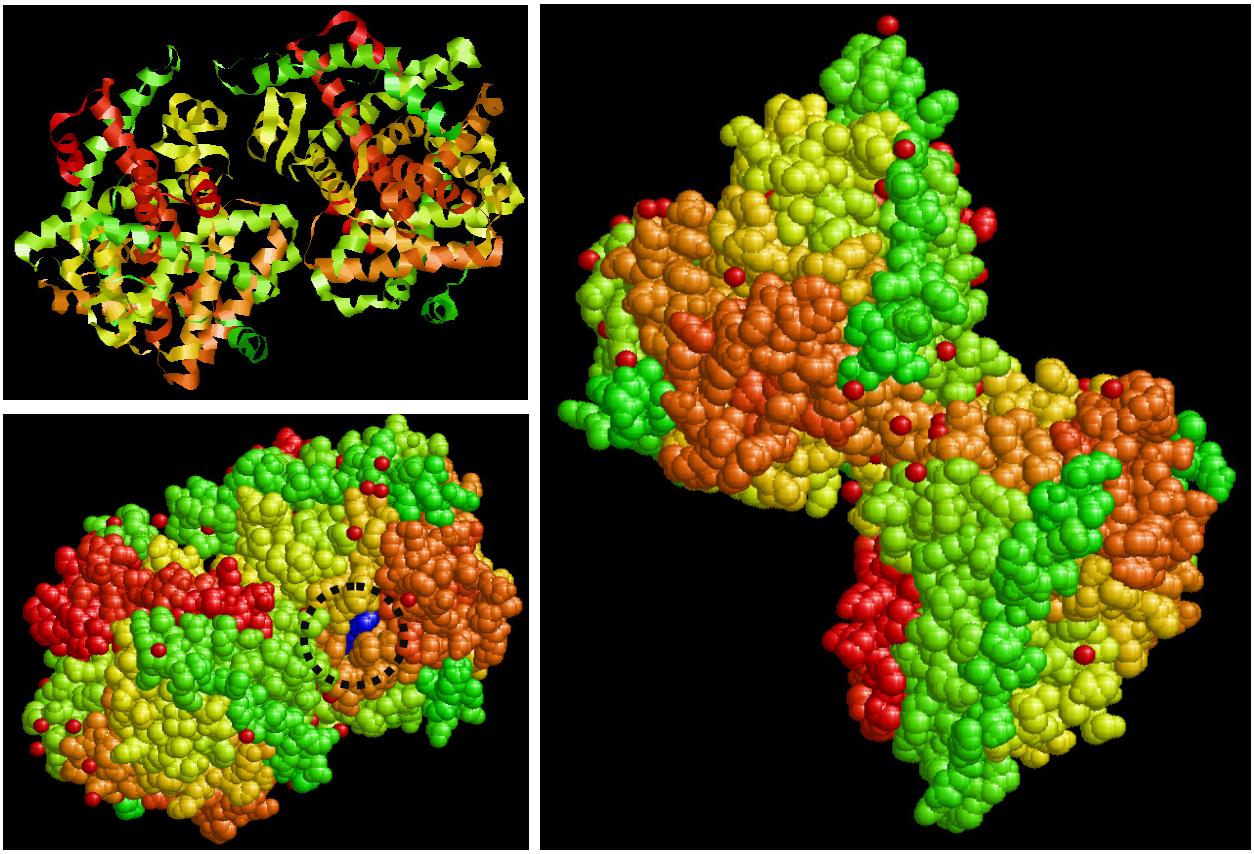

FIGURE 4: Estrogen receptor complexed with isoflavone. Top left and right figure shows Estrogen receptor without its ligand bound. Bottom left figure shows Estrogen receptor with Isoflavone bound tightly in the groove (circled with dotted black line) created by the active site.

PatchDock is a computational tool that we used to measures binding affinity between the protein and the small molecule/ligand Isoflavone. The patchDock results were compared to control ligand (Estradiol) and the following results were found-

Ligand type	Score	Area	ACE	Transformation
Isoflavone	4442	502.90	-118.88	1.56, 0.18, 2.63, 49.94, -7.63, 3.63
Estradiol (control)	2912	302.30	-304.04	0.79 1.04 -1.06 28.22 8.47 -2.88

TABLE 1: PatchDock results for Isoflavone-Estrogen receptor binding

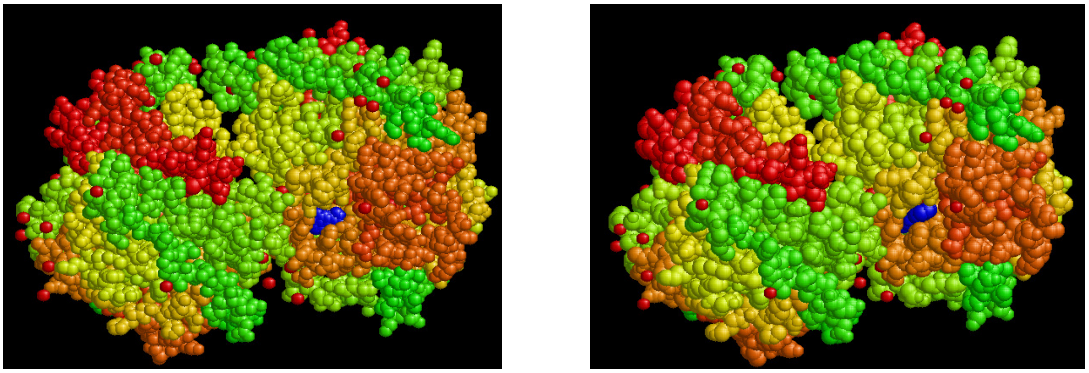

FIGURE 5: Juxtaposition of control and predicted drug binding. The figure on the left shows binding for natural substrate (marked in blue) and the right one shows binding of isoflavone.

Parameters analyzed in PatchDock computational tool are as follows-

- Score: Geometric shape complementarity score (Duhovny D NR *et al.* 2002) [15]. The solutions are sorted according to this score.
- Area: Approximate interface area of the complex.
- ACE: Atomic contact energy according to Zhang *et al.* 1997 [16].
- Transformation: 3D transformation on the ligand molecule. 3 rotational angles and 3 translational parameters.

PocketFinder computational tool was used to find active site within the protein strudture. Although the tool gives positive results for few potential active site, only the predicted site 4 (**Figure-7**) shows significance within confidence interval. The active site correlates excellently to the one cleft where isoflavone binds (**FIGURE-6**).. Thus, it confirms tight binding of isoflavone in the exact groove where estrogen binds in its receptor.

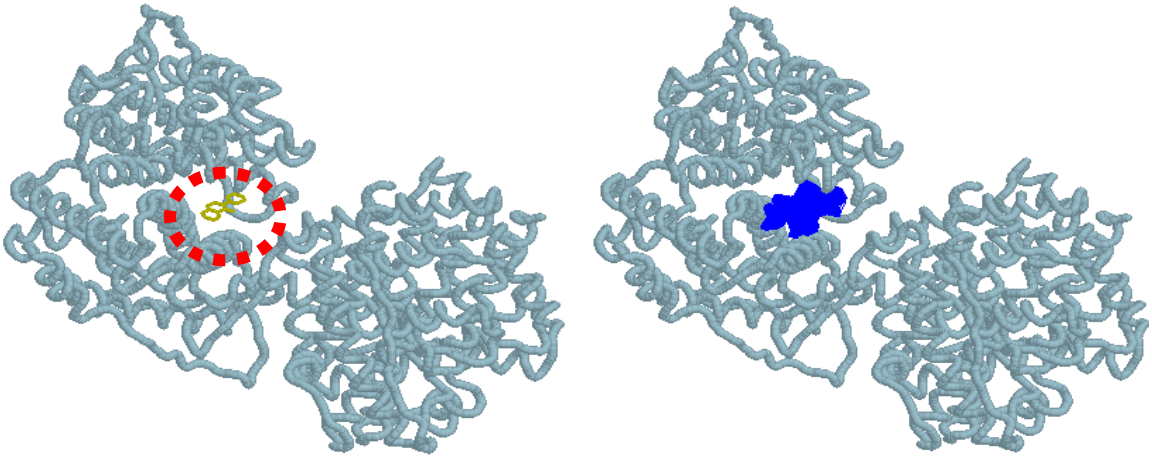

FIGURE 6: Pocket-Finder results for surface cleft identification. Binding site of the ligand Isoflavone (Left), as predicted by the tool. The right figure shows binding cleft for natural substrate Estrogen.

Amino acids residues interacting at the active site are- Gln371, Gln374, Asn375, Arg428, His429, Ala432, Val433, Leu435, His439, Val391, Asn392, Ala393, Glu394, Val395, Arg396, Leu398, Tyr401, Phe405, Gln408, Gln409.

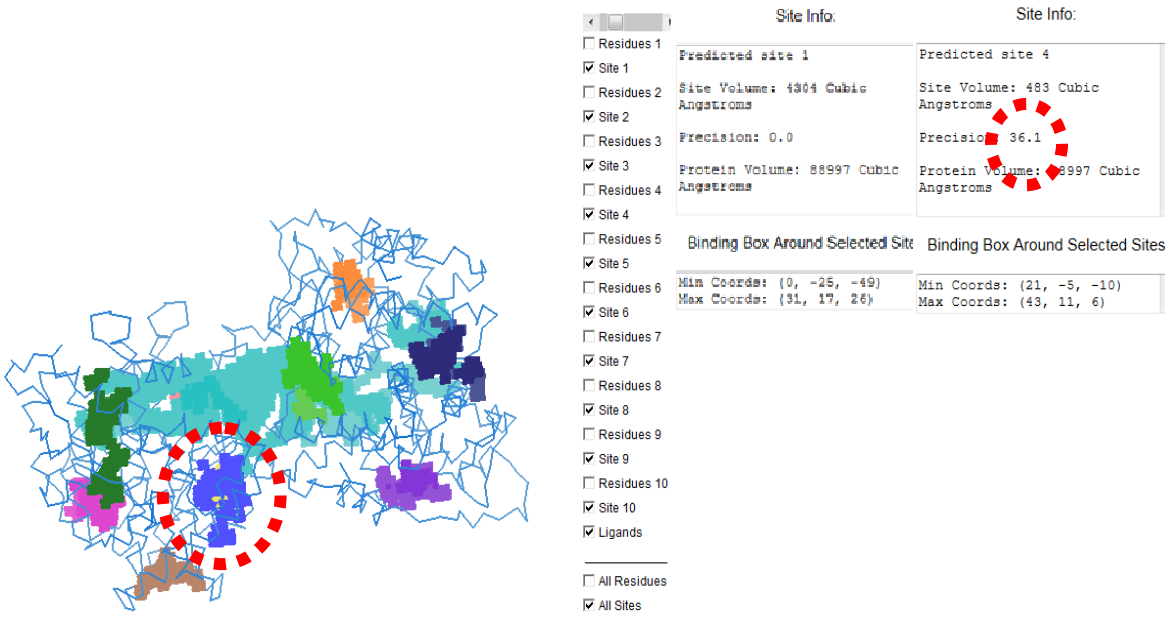

FIGURE 7: Pocket-Finder results for cleft verification. The results show that for predicted cleft 1 (marked with Cyan patch) the precision was 0.00 whereas for surface cleft 4 (marked with blue patch, circled with dotted red line) the precision was 36.1 which fits well in the range of the confidence interval.

4. DISCUSSION

Herbal plants have been one of the most important natural sources of isolating new active products. *Ochna squarrosa* has for long been identified to decrease menstrual pain, bleeding and muscle cramp (Vissandjee B *et al.* 1997) [18]. Here, our results show that the *O. squarrosa* has an active product which is Isoflavone (or its derivative) and it binds to estrogen receptor as an agonist which in result binds to gene regulatory elements responsible for alleviating the menstrual pain.

Although, a counter-current chromatography could have doubtlessly proved the presence of the active product (Renmin Liu *et al.* 2004) [17], our chemical evidences were sufficient enough for proving the presence of Isoflavone or its derivative conclusively. Besides, case studies of the closely related species such as Ochna jabotapita reveal the presence of similar active product.

Distinguishing between energy and binding parameters between agonist and antagonist has been the Holy Grail in structure based drug designing. However, based on the phenotypic effect it is clear that the herbal extract must enhance the effect of the receptor instead of reducing it. This is even clearer from our score data in **TABLE 1** where the score of the small molecule is fairly larger than the natural ligand.

The shape similarity between isoflavone ligand and estrogen was remarkable which gave us an initial indication about the ligand's target protein. The score for docked ligand and the estrogen receptor was significant enough. The score we found for an unbound ligand was almost the half the value that we derived. The high 'Area' of interaction also reveals tight binding. The ligand was bound well inside the active site cleft and no nonspecific interaction was found. Therefore, the binding of isoflavone to the estrogen receptor definitely indicates potential of a predictive drug. PocketFinder results scoringconfirmed that isoflavone and estrogrn share the same binding cleft (**FIGURE 6 and FIGURE 7**). Isoflavone, as it seems to accelerate estrogen activity, works as an agonist of estrogen for the receptor.

Modern rational drug designing belongs to a twofold process. At first, potential hit are generated using approaches as carried out here. However, irrespective of values hits have to be analyzed furthermore in the molecular level physically. Despite our significant scoring values we believe the drug protein interaction will have to be further investigated physically.

Many drugs that we consume today sprang from ancient herbal science. However, use of many traditional herbal plants has been questioned by skeptics. Few hardliner, calling into question of herbal practices, have even gone into the degree of calling some of the ancient 'herbal science' a 'voodoo science'. Others, more measured critics have termed most uses to have a mere mollifying effect rather than any active drug response. In this context, predicting mode of action of herbal plants certainly is a happening field that concerns issues starting from religious to purely scientific. Our study on this particular plant was based on local observation that it works well to cure menstrual complexities. We believe our results underpin the scientific background behind the use with verifiable proof.

Our study here should give us better insight about how the traditional use of *O. squarrosa* is riveted well inside science rather than folklore. We hope, this study would help in designing a more efficient rational drug in the future and also would help us collect more natural products from the plant concerned. Hence, we hope the study will assist us to understand the beauty underneath natural products, to unlock the mystery of nature itself.

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