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RESEARCH ARTICLE

In Silico Elucidation of the Plausible Inhibitory Potential of *Withaferin A* of *Withania Somnifera* Medicinal Herb Against Breast Cancer Targeting Estrogen Receptor

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Abstract: ***Background*:** Estrogen receptors (ER) are members of the nuclear intracellular receptors family. ER once activated by estrogen, it binds to DNA via translocating into the nucleus and regulates the activity of various genes. Withaferin A (WA) – an active compound of a medicinal plant *Withania Somnifera* was reported to be a very effective anti-cancer agent and some of the recent studies has demonstrated that WA is capable of arresting the development of breast cancer via targeting estrogen receptor.

***Objective*:** The present study is aimed at understanding the molecular level interactions of ER and Tamoxifen in comparison to Withaferin A using *In-silico* approaches with emphasis on Withaferin A binding capability with ER in presence of point mutations which are causing *de novo* drug resistance to existing drugs like Tamoxifen.

***Methods*:** Molecular modeling and docking studies were performed for the Tamoxifen and Withaferin A with the Estrogen receptor. Molecular docking simulations of estrogen receptor in complex with Tamoxifen and Withaferin A were also performed.

***Results*:** Amino acid residues, Glu353, Arg394 and Leu387 was observed as crucial for binding and stabilizing the protein-ligand complex in case of Tamoxifen and Withaferin-A. The potential of Withaferin A to overcome the drug resistance caused by the mutations in estrogen receptor to the existing drugs such as Tamoxifen was demonstrated.

***Conclusion*:** *In-silico* analysis has elucidated the binding mode and molecular level interactions which are expected to be of great help in further optimizing Withaferin A or design / discovery of future breast cancer inhibitors targeting estrogen receptor.

Keywords: *Withania Somnifera*, Estrogen receptor, Tamoxifen, *Withaferin A*, molecular docking, molecular dynamics simulations.

1. INTRODUCTION

Estrogen receptors (ER) are members of the nuclear intracellular receptors family [1]. The binding of estrogens causes physiological effects on ER. α and β are two types of estrogen receptors encoded by *ESR1* and *ESR2* genes respectively. ERα regulates the maintenance and differentiation of cardiovascular, neural, skeletal, and reproductive tissues [2]. ERs form dimers when estrogen hormone binds to it, and since ERα and β forms are co-expressed usually, they may form either homodimers or heterodimers as ERα (αα) / ERβ (ββ) or ERαβ (αβ) respectively.

Compounds that are capable of modulating the transcriptional activity of ERα are currently being used to treat diseases, such as cardiovascular, osteoporosis, and breast cancer

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[3]. Synthetic ligands, such as active metabolite of tamoxifen -4-hydroxytamoxifen (OHT) which function as antagonists are one among the growing class of Selective Estrogen Receptor Modulator (SERM) molecules [4, 5].

According to the reported crystal structure, OHT is bound to ER *via* forming a hydrogen bonding between phenolic hydroxyl and structurally conserved water with the side chains of Glu353 and Arg394 in between helices 3 and 6 of ER. Whereas, the OHT C ring was reported to be forming van der Waals interactions with the side chains of Met343, Leu346, Thr347, Ala350, Trp383, Leu384, Leu387, and Leu525 residues. The flexible side chain dimethylaminoethyl region is stabilized by a salt bridge between the β-carboxylate of Asp351 and the dimethylamino group of the side chain along with van der Waals interactions with Thr347, Ala350, and Trp383. And residues between 536 and 544 in ER were reported to be crucial role players in recruiting and binding of the tamoxifen to the ER ligand-binding domain [6].

SERMs or Aromatase Inhibitors (AIs) for estrogen deprivation is an important endocrine therapy modality for ER-positive breast cancer patients. Due to intrinsic resistance about ~40% of patients show low / no response from first-line endocrine therapy [7]. Although several theories have been associated with endocrine resistance, the mode of action for resistance has not been identified yet with clinical significance [8,9,10]. Recently, particular interest has been drawn towards mutations in the ERα and ESR1 genes, as a plausible mechanism for endocrine resistance in breast cancer. Massive parallel Next Generation Sequencing (NGS) studies revealed that patients treated with endocrine agents are enriched with these mutations while these variants are absent or present at a very low prevalence in primary tumor tissue [11, 12].

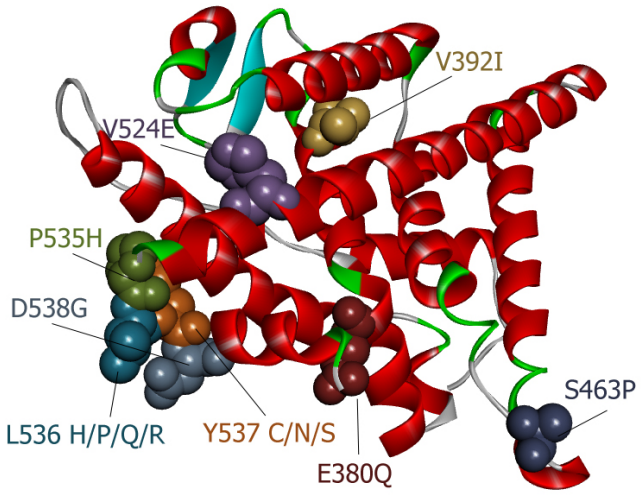

Fig. (1). 3D structure of the estrogen receptor (PDB ID: 3ERD) showing the ribbons and the mutated residues are shown in the spacefilling model. (*A higher resolution/colour version of this figure is available in the electronic copy of the article*).

NGS based studies on breast tissues also revealed that somatic ESR1 mutations (Fig. **1**) were reported in metastatic lesions far more frequently than speculated. Some preclinical studies have demonstrated that cell lines transfected with an E380Q, S463P, L536Q, Y537S / N / C or D538G mutations in ESR1 gene were observed to be active in the absence of estrogen [11, 13-18]. Toy and colleagues [11] reported that D538G and Y537S mutations in ESR1 are predominant in ER-positive patients' metastatic tissues who had received at least three months of endocrine therapy. Furthermore, Jeselsohn *et al.* [12] detected ESR1mutations (Y537N/C/S and D538G) in about 12% of ER-positive patients with metastatic tumors. On one hand, it is yet to be revealed that what's the impact of different ESR1 mutations in distinctive phenotypes, while on the other hand, very little functional clinical study evaluation on ESR1 mutations especially K303R, V524E, P535H, L536H/P/R and their role in endocrine resistance has been performed at all.

Withania Somnifera, also known as Ashwagandha belonging to family Solanaceae [19], genus Withania with 23 species is an important medicinal plant that was in use for more than 3000 years for its Ayurvedic and indigenous medicine benefits. It is most commonly found in drier parts of subtropical and tropical zones. Withanolides, which are part of 35 chemical constituents are identified as responsible for its extraordinary medicinal properties [20].

Saponins containing an additional acyl group (sitoindoside VII and VIII), alkaloids (anferine, isopellertierine), withanoloides with glucose at carbon 27 (sitonidoside XI and X) and steroidal lactones (withanolides, withaferins) are some of the important biologically active chemical constituents. Withaferin A (WA) is one of the most important withanolides, which is attributed to much of Ashwagandha's pharmacological properties. The biological activities of *W. somnifera* are anxiolytic, antidepressant, antifungal [21], antimicrobial [22], antimalarial [23], apoptotic [24], chondroprotective [25], cardioprotective [26], immunomodulatory [27], and neuroprotective [28], anti-inflammatory *via* COX-2 inhibition [29], and enhance memory and learning capabilities in patients with Alzheimer's disease (AD) [30]. It also holds the capability of influencing the central nervous system (CNS) *via* modulating neurotransmitter receptors [31].

It has been reported that WA can stimulate tumor cell apoptosis in MCF-7 breast cancer cells [32,33]. Stan *et al.* [34] showed that WA causes cell cycle arrest in human breast cancer cell lines MDA-MB-231 and MCF-7 at their G2 and M phases. Thaiparambil *et al.* [35] studies shown that the administration of WA 4mg/kg *via* intraperitoneal for 30 days in 4T1 mouse breast reduces the intensity of tumor growth and Hahm *et al.* [36] studies revealed that 100μg/mouse intraperitoneal administration of WA for 196 days in MMTV-neu (breast) causes decrease in tumor burden at both microscopic and macroscopic level. In another study, Hahm *et al.* [37] systematically determined the role of p53 and ER-α in pro-apoptotic response to WA using T47D, MCF-7 and ER-α overexpressing MDA-MB-231 cells as a model.

In this scenario, the present study is aimed at revealing the molecular-level interactions of Withaferin A compared to Tamoxifen using *In-silico* approaches with emphasis on Withaferin A binding capability with ER in the presence of point mutations which are causing de novo drug resistance to existing drugs like Tamoxifen.

2. METHODOLOGY

2.1. Software and Program

Schrodinger's maestro [38], Dassault Systèmes BIOVIA Discovery Studio [39], Zinc database [40], PyRx 8.0, Autodock 4.0 [41], Schrodinger's Desmond module [42], Protein Data Bank (PDB) [43] were the major software programs used to retrieve protein [PDB ID: 3ERD] and ligand [ZINC08234189], prepare and execute the virtual screening, molecular modeling, docking and NPT MD simulations using default protocols and parameters of the software programs have been applied similar to the protocol followed elsewhere [44-49]. Briefly, the autodock was run with a grid size of 126Å XYZ coordinates employing a genetic algorithm. Docking snapshots were prepared using BIOVIA Discovery Studio and simulation graphs were generated using Schrodinger's desmond simulation event analysis and simulation interaction diagram modules.

3. RESULTS AND DISCUSSION

3.1. Virtual Screening of Tamoxifen and Withaferin-A with Mutated Versions of ER

In order to understand how the point mutations are affecting the binding capability of Tamoxifen and Withaferin A, we have performed virtual screening of these two ligands over modeled mutated versions D538G, E380Q, L536H, L536P, L536Q, L536R, P535H, S463P, V392I, V524E, Y537C, Y537N and Y537S of ER. Results of this virtual screening in Kcal/mol are tabulated in Table **1**.

From the virtual screening results, it is evident that except L536R, Y537C and Y537S mutations other point mutation versions were showing a decline in binding capabilities, where above-mentioned L536R, Y537C and Y537S mutated ER were observed to be with a similar binding energy of wild type (WT) ER. Whereas in the case of Withaferin-A; E380Q, L536H, L536Q, P535H and V392I mutated versions of ER have shown a slight increase in binding capability and rest of mutated versions were observed to be similar to that of wild type ER.

3.2. Docking of Tamoxifen and Withaferin-A with ER

Molecular modeling and docking studies were performed for the Tamoxifen and Withaferin A with the Estrogen receptor to better understand and reveal the binding energies involved in their complex formation and to know the molecular level interactions responsible for their inhibition. Another purpose of the docking study is also to know the preferred binding site for the compounds in the estrogen receptor. Docking results of Tamoxifen and Withaferin A with ER are tabulated in Table **2**.

Both compounds Tamoxifen and Withaferin A have shown to be successfully docking at the active site of Estrogen receptor with binding energies of -6.64 and-8.27 Kcal/mol respectively. As per the docking results, it was revealed that Withaferin A has a better estimated -8.27 Kcal/mol of binding energy for the Estrogen receptor inhibited complex formation by forming hydrogen bonds with PRO324, ILE326, ILE386, TRP393, ARG394, and LYS449. Withaferin A was also found to be forming Van der Waals interactions with GLU323, PRO325, LEU327, GLU353, HIS356, MET357, LEU387, GLY390 and MET396. Whereas, Tamoxifen has an estimated -6.64 Kcal/mol for the Estrogen receptor by forming hydrogen bonds with GLU323, ILE326, ARG394, LYS449 and PRO325. Tamoxifen was also found to be forming Van der waals interactions with GLU397, PHE445, TRP393, GLY390, MET357, LEU387, GLU353, PRO325 (Fig. **2**). Although residues 536 to 544 were reported to key role players in recognition and binding of tamoxifen according to the reported structure, none of them were observed to interacting with Tamoxifen in our study. However, Glu353 and Leu387 residues which were reported to be forming hydrogen bonding via its phenolic hydroxyl and van der walls interaction respectively with OTH were observed in our study as well. Whereas, Withaferin-A was observed to be interacting with Arg394 and Glu353 and Leu387 via hydrogen bonding and Van der Waals interactions respectively.

3.3. IC50 Prediction

Half maximal (IC50) inhibitory concentration can be used as a parameter towards comparing and evaluating the plausible experimental activity of the compounds. The inhibition constant Ki = IC50/(1+L*/Kd), where L* is the concentration, and Kd the equilibration dissociation constant of ligand equation is used as part of the Autodock program to predict IC50 values. The predicted IC50 value for the Tamoxifen and Withaferin A showed in table 1having an estimated13.50 and 4.36 micromolar respectively. Promisingly good IC50 values have been obtained for the Withaferin A compared to Tamoxifen as a demonstration of its inhibitory potential with estrogen receptor.

3.4. MD Simulations of Estrogen Receptor in Complex with Tamoxifen and Withaferin A

To reveal the stability and conformational changes along with the aim of understanding their underlying molecular interactions at the atomic level of the Estrogen receptor in complex with Tamoxifen and Withaferin A, we have conducted the molecular dynamic simulations for 100 nanoseconds each separately.

We have analyzed the simulation quality parameters before starting analyzing the trajectory of simulation to ensure that the simulation was carried out under our given simulation conditions throughout the simulated time scale. 300 k temperature; pressure at 0 atmospheric and volume of the simulations box is kept at an average of 287500 $Å^3$ throughout the simulated time. As evident in Fig. (**3**), simulation conditions were maintained thoroughly during the entire simulated time of 100 nanoseconds, thus confirming the quality of the simulations carried out.

3.5. Comparative Analysis of the Impact of Protein Stabilizing Capability by Tamoxifen and Withaferin A

As part of the simulation events analysis, we have tried to estimate the protein stabilizing capability by Tamoxifen and Withaferin A via calculating the averaged Root mean square fluctuations (RMSF) of each residue during the simulated timescale of 100 nanoseconds each.

Table 1. Virtual screening results of the Tamoxifen and Withaferin A with mutated versions of Estrogen receptor.

S. No.	Ligand	Target	Binding Energy (Kcal/mol)
1.	Tamoxifen	ER_3ERD_WT	-6.0
2.	Tamoxifen	ER_3ERD_D538G	-5.9
3.	Tamoxifen	ER_3ERD_E380Q	-5.8
4.	Tamoxifen	ER_3ERD_L536H	-5.9
5.	Tamoxifen	ER_3ERD_L536P	-5.9
6.	Tamoxifen	ER_3ERD_L536Q	-5.9
7.	Tamoxifen	ER_3ERD_L536R	-6.0
8.	Tamoxifen	ER_3ERD_P535H	-5.9
9.	Tamoxifen	ER_3ERD_S463P	-5.8
10.	Tamoxifen	ER_3ERD_V392I	-5.9
11.	Tamoxifen	ER_3ERD_V524E	-5.8
12.	Tamoxifen	ER_3ERD_Y537C	-6.0
13.	Tamoxifen	ER_3ERD_Y537N	-5.9
14.	Tamoxifen	ER_3ERD_Y537S	-6.0
15.	Withaferin-A	ER_3ERD_WT	-8.0
16.	Withaferin-A	ER_3ERD_D538G	-8.0
17.	Withaferin-A	ER_3ERD_E380Q	-8.1
18.	Withaferin-A	ER_3ERD_L536H	-8.1
19.	Withaferin-A	ER_3ERD_L536P	-8.0
20.	Withaferin-A	ER_3ERD_L536Q	-8.1
21.	Withaferin-A	ER_3ERD_L536R	-8.0
22.	Withaferin-A	ER_3ERD_P535H	-8.1
23.	Withaferin-A	ER_3ERD_S463P	-8.0
24.	Withaferin-A	ER_3ERD_V392I	-8.1
25.	Withaferin-A	ER_3ERD_V524E	-8.0
26.	Withaferin-A	ER_3ERD_Y537C	-8.0
27.	Withaferin-A	ER_3ERD_Y537N	-8.0
28.	Withaferin-A	ER_3ERD_Y537S	-8.0

Table 2. Docking results of the Tamoxifen and Withaferin A with Estrogen receptor.

S. No.	Compound	Binding Energy (Kcal/mol)	Predicted IC50 values in Micro Molar Units
1.	Tamoxifen	-6.64	13.50 uM
2.	Withaferin A	-8.27	4.36 uM

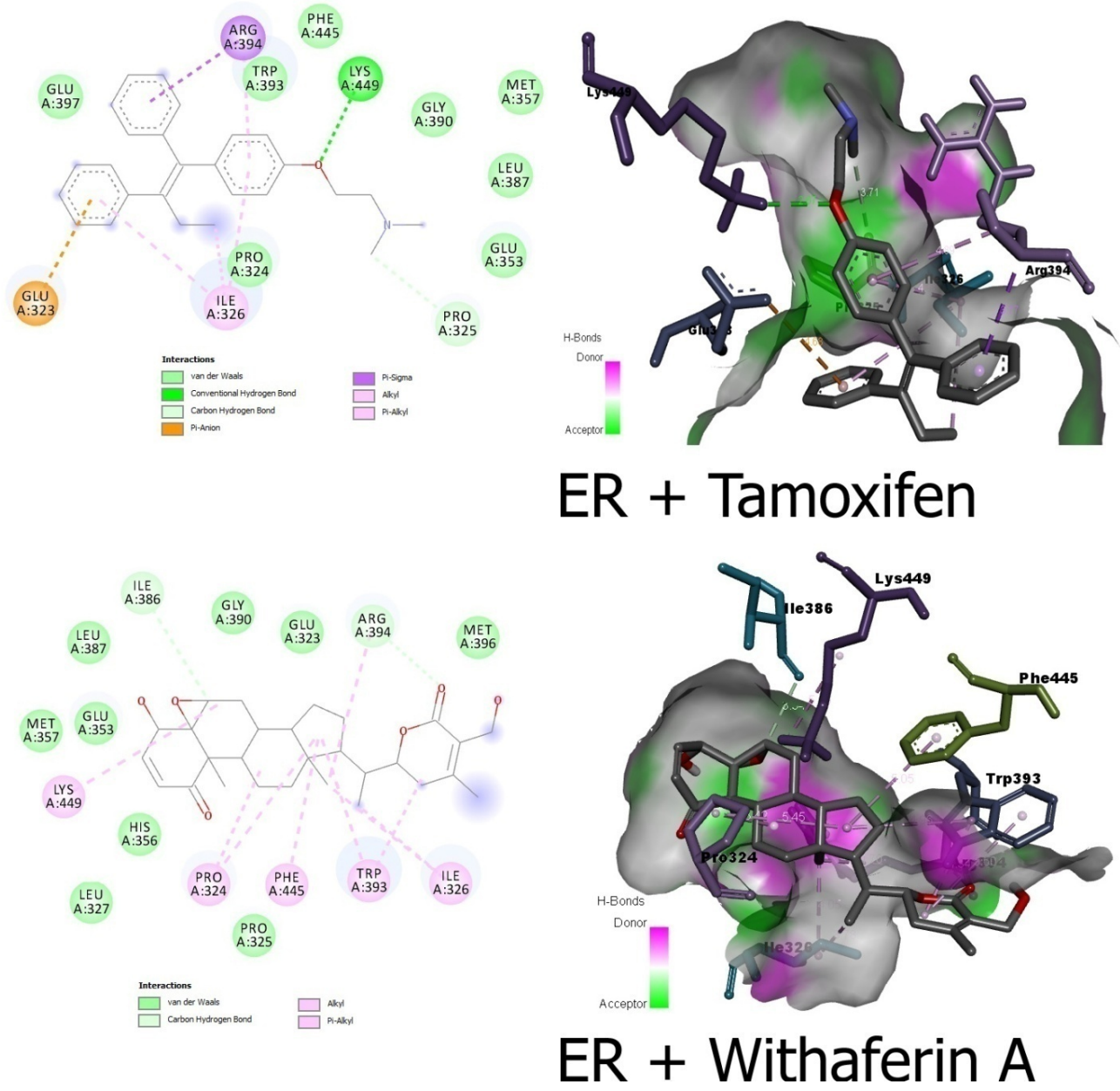

Fig. (2). Docking snapshots of compound 5 with Estrogen receptor showing molecular interaction responsible for the binding. (*A higher resolution/colour version of this figure is available in the electronic copy of the article*).

From the RMSF graph (Fig. **4**), it can be observed that Withaferin A in majority shown much better energy minimizing the effect on estrogen receptor's residues, especially those which are involved in de novo drug resistance compared to tamoxifen. While majority of the residues were quite stable throughout the simulated timescale with an average of not more than 1.50 Å, there are few portions of the protein where fluctuations have been observed up to 3.7 Å. Notably, a small loop of about ten residues between 360 and 370 has shown higher fluctuations in estrogen receptor in the presence of Tamoxifen compared to Withaferin A. Fluctuations among reported mutations were observed for Tamoxifen and Withaferin-A respectively as 1.0 Å, 1.2 Å for E380Q; 2.0 Å, 0.9 Å for V392I; 1.3 Å, 1.1 Å for S463P; 1.2 Å, 0.9 Å for V524E; 1.3 Å, 1.7 Å for P535H; 1.0 Å, 0.9 Å for L536 H/P/Q/R; 1.5 Å, 0.6 Å for Y537 C/N/S; 1.2 Å, 0.6 Å for D538. From the RMSF graph and above observed values, it can be inferred that Withaferin A has potential to minimize the activity of the protein by restricting the residues movement especially those involved in causing drug resistance (E380Q, S463P, L536Q, Y537S/N/C, D538G, V524E, P535H, L536H/P/R) to overcome the *de novo drug resistance* even at the portions of the proteins where the responsible mutations are present in the targeted estrogen receptor compared to Tamoxifen.

3.6. Ligand Properties Profile in Complex with Estrogen Receptor During MD Simulations

As part of the simulations event analysis, we have analyzed Ligand properties such as RMSD, intra molecular H-bonds; the radius of gyration; polar surface area, molecular

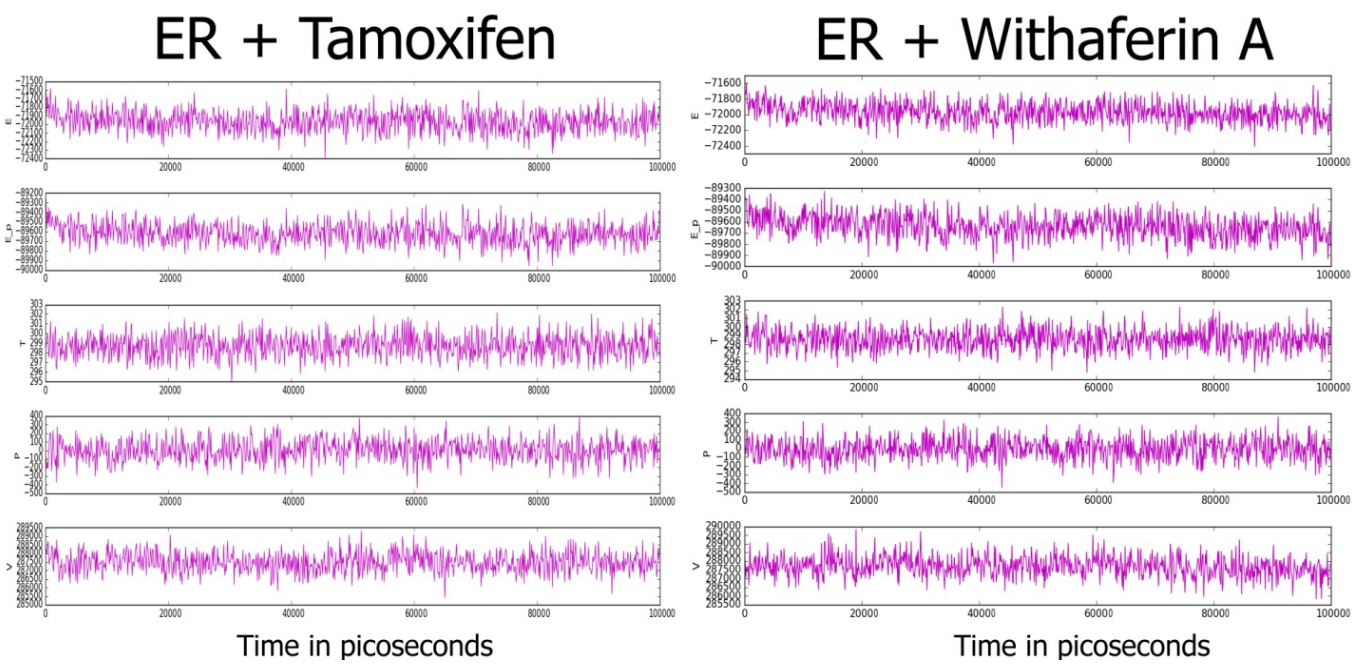

Fig. (3). Simulation Quality analysis of the Estrogen receptor in complex with Tamoxifen (Left) and Withaferin A (Right). (*A higher resolution/colour version of this figure is available in the electronic copy of the article*).

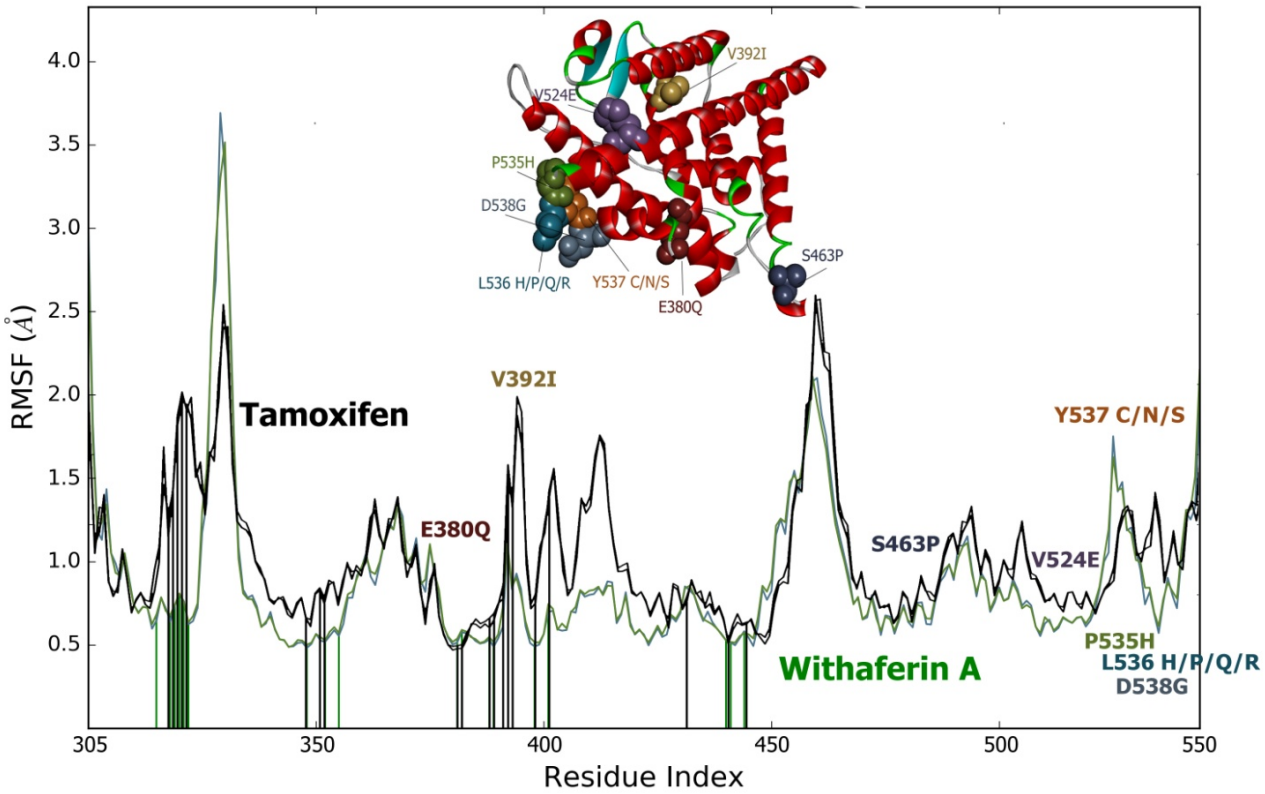

Fig. (4). Root means square fluctuation (RMSF) analysis during simulation of the Estrogen receptor in complex with Tamoxifen (black) and Withaferin A (green). (*A higher resolution/colour version of this figure is available in the electronic copy of the article*).

surface area and solvent accessible surface area of the ligand during the simulated length of time scale. As evident with Fig. (**5**), Tamoxifen RMSD was observed to be fluctuating between 0.3 to 2.0 with a mean of 1.5 Å which is good enough to state that the ligand Tamoxifen is quite stable inside the active site of the ER throughout the simulated time. Whereas, Withaferin A was found to be fluctuating between 0.5 to 2.4 with an average of 1.6 Å, which is very well within

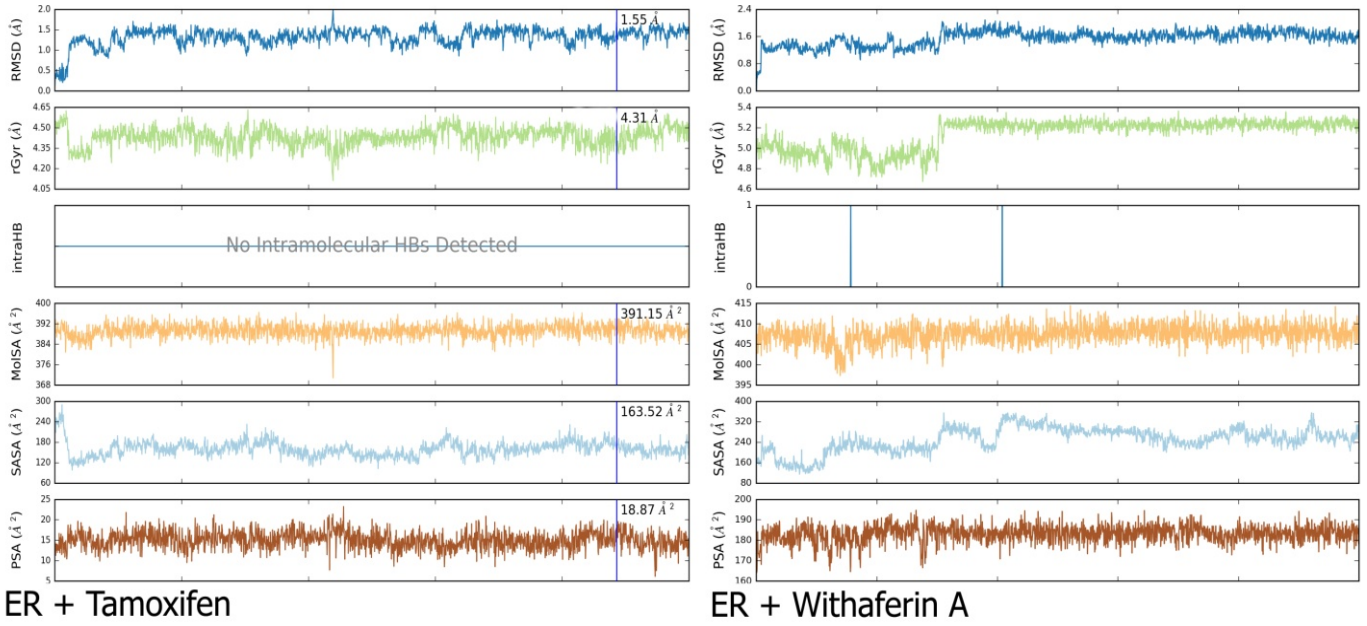

Fig. (5). Tamoxifen (Left panel) and Withaferin A (right panel) properties analysis during simulated timescale. (*A higher resolution/colour version of this figure is available in the electronic copy of the article*).

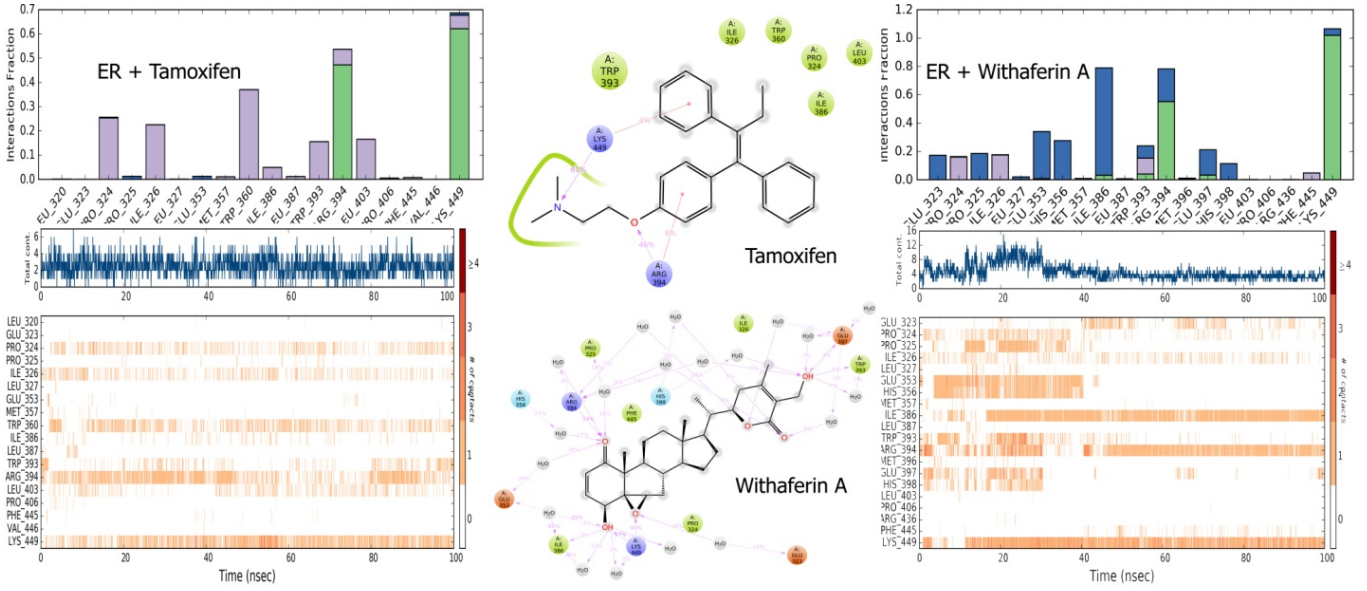

Fig. (6). Protein interactions with the ligands Tamoxifen (left) and Withaferin A (right) as monitored throughout the simulated timescale. (*A higher resolution/colour version of this figure is available in the electronic copy of the article*).

the stabilizing range. Although Withaferin A has shown a bit higher RMSD compared to Tamoxifen it's quite stable after the initial 30 ns comparatively.

As the simulation progresses, the Withaferin A was found to be quite stable in its overall size as evident with its radius of gyration with an average of5.3 Å after the first 30ns. Whereas, Tamoxifen has shown fluctuations throughout simulation with an average of 4.3 Å. No intramolecular hydrogen bonds were found to be forming within Tamoxifen but a couple of intramolecular hydrogen bonds were observed within Withaferin A which are thought to be responsible for stability the ligand size as observed in the radius of gyration graph. Solvent accessible surface area (SASA), Molecular surface (MolSA) area along with polar surface area (PSA) which are some of the important indicators of hydrophobicity of the compounds for oral bioavailability was found to be maintaining an average of 150, 392 and 15 $Å^2$ for Tamoxifen and 410, 300, 180 Å2 for Withaferin A respectively.

3.7. Molecular Interactions of Estrogen Receptor in Complex with Tamoxifen and Withaferin A During MD Simulations

During molecular interactions analysis between Estrogen receptor and Tamoxifen and Withaferin A, it was revealed that about 18 contacts found in between Estrogen receptor and Tamoxifen in total among with up to 6 interactions during the simulation with two hydrogen bond were observed with ARG394 and LYS449 residues. Hydrophobic contacts were found to forming with residues PRO324, ILE326, MET357, TRP360, ILE386, LEU387, TRP393, ARG394, LEU403, PRO406, PHE445 and LYS449. Several H-bonds were observed to be stabilized via forming water brides with the residues LEU320, GLU323, PRO325, LEU327, GLU353, PRO406, PHE445 and VAL446. About twenty contacts observed between Estrogen receptor and Withaferin A in total among which eight H-bonds were observed with MET357, ILE386, LEU387, TRP393, ARG394, MET396, GLU397 and LYS449. Hydrophobic contacts were found to forming with residues PRO324, ILE326, TRP393 and PHE445. Several hydrogen bonds were observed facilitated by water brides with the residues GLU323, PRO325, LEU327, GLU353, HIS356, MET357, ILE386, TRP393, ARG394, GLU397, HIS398 and LYS449. In both cases, and the majority of other contacts were found to be hydrophobic contacts followed by water bridging and ionic bonds. Apart from the above molecular interactions, some positive and negative charges are thought to have a crucial role in binding Tamoxifen and Withaferin-A towards stabilizing the protein-ligand complexes.

CONCLUSION

In the present work, we have demonstrated the ability along with the plausible mode of action of Withaferin A – an active constituent of *Withania Somnifera* medicinal herb as a potential breast cancer inhibitor targeting the estrogen receptor. Our *In-silico* analysis has elucidated the binding mode and molecular level interactions which are expected to be of great help in further optimizing this compound or design/ discovery of future breast cancer inhibitors targeting the estrogen receptor. Among many of the previously reported important residues, Glu353, Arg394 and Leu387 was observed to crucial for binding and stabilizing the protein-ligand complex in case of Tamoxifen and Withaferin-A via hydrogen bondings/ Van der Waals interactions. Our report is of crucial importance in elucidating the potential of Withaferin A to overcome the drug resistance caused by the reported mutations in the estrogen receptor to the existing drugs such as Tamoxifen. This study also demonstrated that estrogen receptor could be of a potential drug target for Withaferin A. Further studies to validate these results using *in-vitro/in-vivo* studies would be invaluable in designing novel scaffolds of inhibitors against breast cancer targeting estrogen receptor.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.

HUMAN AND ANIMAL RIGHTS

No Animals/Humans were used for studies that are base of this research.

CONSENT FOR PUBLICATION

Not applicable.

AVAILABILITY OF DATA AND MATERIALS

Not applicable.

FUNDING

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CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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REFERENCES

[1] Tsai, M.J.; O'Malley, B.W. Molecular mechanisms of action of steroid/thyroid receptor superfamily members. *Annu. Rev. Biochem.,* **1994**, *63*, 451-486. http://dx.doi.org/10.1146/annurev.bi.63.070194.002315 PMID: 7979245

[2] Korach, K.S. Insights from the study of animals lacking functional estrogen receptor. *Science,* **1994**, *266*(5190), 1524-1527. http://dx.doi.org/10.1126/science.7985022 PMID: 7985022

[3] Jordan, V.C.; Gradishar, W.J. Molecular mechanisms and future uses of antiestrogens. *Mol. Aspects Med.,* **1997**, *18*(3), 167-247. http://dx.doi.org/10.1016/S0098-2997(96)00015-5 PMID: 9220447

[4] Grainger, D.J.; Metcalfe, J.C. Tamoxifen: teaching an old drug new tricks? *Nat. Med.,* **1996**, *2*(4), 381-385. http://dx.doi.org/10.1038/nm0496-381 PMID: 8597938

[5] Grese, T.A.; Sluka, J.P.; Bryant, H.U.; Cullinan, G.J.; Glasebrook, A.L.; Jones, C.D.; Matsumoto, K.; Palkowitz, A.D.; Sato, M.; Termine, J.D.; Winter, M.A.; Yang, N.N.; Dodge, J.A. Molecular determinants of tissue selectivity in estrogen receptor modulators. *Proc. Natl. Acad. Sci. USA,* **1997**, *94*(25), 14105-14110. http://dx.doi.org/10.1073/pnas.94.25.14105 PMID: 9391160

[6] Shiau, A.K.; Barstad, D.; Loria, P.M.; Cheng, L.; Kushner, P.J.; Agard, D.A.; Greene, G.L. The structural basis of estrogen receptor/coactivator recognition and the antagonism of this interaction by tamoxifen. *Cell,* **1998**, *95*(7), 927-937. http://dx.doi.org/10.1016/S0092-8674(00)81717-1 PMID: 9875847

[7] Pritchard, K.I. Endocrine therapy: is the first generation of targeted drugs the last? *J. Intern. Med.,* **2013**, *274*(2), 144-152. http://dx.doi.org/10.1111/joim.12065 PMID: 23844917

[8] Osborne, C.K.; Schiff, R. Mechanisms of endocrine resistance in breast cancer. *Annu. Rev. Med.,* **2011**, *62*, 233-247. http://dx.doi.org/10.1146/annurev-med-070909-182917 PMID: 20887199

[9] De Laurentiis, M.; Arpino, G.; Massarelli, E.; Ruggiero, A.; Carlomagno, C.; Ciardiello, F.; Tortora, G.; D'Agostino, D.; Caputo, F.; Cancello, G.; Montagna, E.; Malorni, L.; Zinno, L.; Lauria, R.; Bianco, A.R.; De Placido, S. A meta-analysis on the interaction between HER-2 expression and response to endocrine treatment in advanced breast cancer. *Clin. Cancer Res.,* **2005**, *11*(13), 4741-4748. http://dx.doi.org/10.1158/1078-0432.CCR-04-2569 PMID: 16000569

[10] Amir, E.; Miller, N.; Geddie, W.; Freedman, O.; Kassam, F.; Simmons, C.; Oldfield, M.; Dranitsaris, G.; Tomlinson, G.; Laupacis, A.; Tannock, I.F.; Clemons, M. Prospective study evaluating the

impact of tissue confirmation of metastatic disease in patients with breast cancer. *J. Clin. Oncol.,* **2012**, *30*(6), 587-592. http://dx.doi.org/10.1200/JCO.2010.33.5232 PMID: 22124102

[11] Toy, W.; Shen, Y.; Won, H.; Green, B.; Sakr, R.A.; Will, M.; Li, Z.; Gala, K.; Fanning, S.; King, T.A.; Hudis, C.; Chen, D.; Taran, T.; Hortobagyi, G.; Greene, G.; Berger, M.; Baselga, J.; Chandarlapaty, S. ESR1 ligand-binding domain mutations in hormone-resistant breast cancer. *Nat. Genet.,* **2013**, *45*(12), 1439-1445. http://dx.doi.org/10.1038/ng.2822 PMID: 24185512

[12] Jeselsohn, R.; Yelensky, R.; Buchwalter, G.; Frampton, G.; Meric-Bernstam, F.; Gonzalez-Angulo, A.M.; Ferrer-Lozano, J.; Perez-Fidalgo, J.A.; Cristofanilli, M.; Gómez, H.; Arteaga, C.L.; Giltnane, J.; Balko, J.M.; Cronin, M.T.; Jarosz, M.; Sun, J.; Hawryluk, M.; Lipson, D.; Otto, G.; Ross, J.S.; Dvir, A.; Soussan-Gutman, L.; Wolf, I.; Rubinek, T.; Gilmore, L.; Schnitt, S.; Come, S.E.; Pusztai, L.; Stephens, P.; Brown, M.; Miller, V.A. Emergence of constitutively active estrogen receptor-α mutations in pretreated advanced estrogen receptor-positive breast cancer. *Clin. Cancer Res.,* **2014**, *20*(7), 1757-1767. http://dx.doi.org/10.1158/1078-0432.CCR-13-2332 PMID: 24398047

[13] Weis, K.E.; Ekena, K.; Thomas, J.A.; Lazennec, G.; Katzenellenbogen, B.S. Constitutively active human estrogen receptors containing amino acid substitutions for tyrosine 537 in the receptor protein. *Mol. Endocrinol.,* **1996**, *10*(11), 1388-1398. PMID: 8923465

[14] Zhang, Q.X.; Borg, A.; Wolf, D.M.; Oesterreich, S.; Fuqua, S.A. An estrogen receptor mutant with strong hormone-independent activity from a metastatic breast cancer. *Cancer Res.,* **1997**, *57*(7), 1244-1249. PMID: 9102207

[15] Merenbakh-Lamin, K.; Ben-Baruch, N.; Yeheskel, A.; Dvir, A.; Soussan-Gutman, L.; Jeselsohn, R.; Yelensky, R.; Brown, M.; Miller, V.A.; Sarid, D.; Rizel, S.; Klein, B.; Rubinek, T.; Wolf, I. D538G mutation in estrogen receptor-α: A novel mechanism for acquired endocrine resistance in breast cancer. *Cancer Res.,* **2013**, *73*(23), 6856-6864. http://dx.doi.org/10.1158/0008-5472.CAN-13-1197 PMID: 24217577

[16] Robinson, D.R.; Wu, Y.M.; Vats, P.; Su, F.; Lonigro, R.J.; Cao, X.; Kalyana-Sundaram, S.; Wang, R.; Ning, Y.; Hodges, L.; Gursky, A.; Siddiqui, J.; Tomlins, S.A.; Roychowdhury, S.; Pienta, K.J.; Kim, S.Y.; Roberts, J.S.; Rae, J.M.; Van Poznak, C.H.; Hayes, D.F.; Chugh, R.; Kunju, L.P.; Talpaz, M.; Schott, A.F.; Chinnaiyan, A.M. Activating ESR1 mutations in hormone-resistant metastatic breast cancer. *Nat. Genet.,* **2013**, *45*(12), 1446-1451. http://dx.doi.org/10.1038/ng.2823 PMID: 24185510

[17] Li, S.; Shen, D.; Shao, J.; Crowder, R.; Liu, W.; Prat, A.; He, X.; Liu, S.; Hoog, J.; Lu, C.; Ding, L.; Griffith, O.L.; Miller, C.; Larson, D.; Fulton, R.S.; Harrison, M.; Mooney, T.; McMichael, J.F.; Luo, J.; Tao, Y.; Goncalves, R.; Schlosberg, C.; Hiken, J.F.; Saied, L.; Sanchez, C.; Giuntoli, T.; Bumb, C.; Cooper, C.; Kitchens, R.T.; Lin, A.; Phommaly, C.; Davies, S.R.; Zhang, J.; Kavuri, M.S.; McEachern, D.; Dong, Y.Y.; Ma, C.; Pluard, T.; Naughton, M.; Bose, R.; Suresh, R.; McDowell, R.; Michel, L.; Aft, R.; Gillanders, W.; DeSchryver, K.; Wilson, R.K.; Wang, S.; Mills, G.B.; Gonzalez-Angulo, A.; Edwards, J.R.; Maher, C.; Perou, C.M.; Mardis, E.R.; Ellis, M.J. Endocrine-therapy-resistant ESR1 variants revealed by genomic characterization of breast-cancer-derived xenografts. *Cell Rep.,* **2013**, *4*(6), 1116-1130. http://dx.doi.org/10.1016/j.celrep.2013.08.022 PMID: 24055055

[18] Pakdel, F.; Reese, J.C.; Katzenellenbogen, B.S. Identification of charged residues in an N-terminal portion of the hormone-binding domain of the human estrogen receptor important in transcriptional activity of the receptor. *Mol. Endocrinol.,* **1993**, *7*(11), 1408-1417. PMID: 8114756

[19] Bown, D. *Encyclopedia of herbs and their uses*; RD Press: Montréal, **1995**.

[20] Uddin, Q.; Samiulla, L.; Singh, V.K.; Jamil, S.S. Phytochemical and pharmacological profile of *Withania Somnifera* Dunal: A review. *J. Appl. Pharm. Sci.,* **2012**, *2*, 170-175.

[21] Bhattacharya, S.K.; Bhattacharya, A.; Sairam, K.; Ghosal, S. Anxiolytic-antidepressant activity of *Withania somnifera* glycowithanolides: an experimental study. *Phytomedicine,* **2000**, *7*(6), 463-469. http://dx.doi.org/10.1016/S0944-7113(00)80030-6 PMID: 11194174

[22] Girish, K.S.; Machiah, K.D.; Ushanandini, S.; Harish Kumar, K.; Nagaraju, S.; Govindappa, M.; Vedavathi, M.; Kemparaju, K. Antimicrobial properties of a non-toxic glycoprotein (WSG) from *Withania somnifera* (Ashwagandha). *J. Basic Microbiol.,* **2006**, *46*(5), 365-374. http://dx.doi.org/10.1002/jobm.200510108 PMID: 17009292

[23] Dikasso, D.; Makonnen, E.; Debella, A.; Abebe, D.; Urga, K.; Makonnen, W.; Melaku, D.; Kassa, M.; Guta, M. Anti-malarial activity of *withania somnifera* L. Dunal extracts in mice. *Ethiop. Med. J.,* **2006**, *44*(3), 279-285. PMID: 17447395

[24] Senthilnathan, P.; Padmavathi, R.; Banu, S.M.; Sakthisekaran, D. Enhancement of antitumor effect of paclitaxel in combination with immunomodulatory Withania somnifera on benzo(a)pyrene induced experimental lung cancer. *Chem. Biol. Interact.,* **2006**, *159*(3), 180-185. http://dx.doi.org/10.1016/j.cbi.2005.11.003 PMID: 16375880

[25] Sumantran, V.N.; Kulkarni, A.; Boddul, S.; Chinchwade, T.; Koppikar, S.J.; Harsulkar, A.; Patwardhan, B.; Chopra, A.; Wagh, U.V. Chondroprotective potential of root extracts of *Withania somnifera* in osteoarthritis. *J. Biosci.,* **2007**, *32*(2), 299-307. http://dx.doi.org/10.1007/s12038-007-0030-3 PMID: 17435322

[26] Hamza, A.; Amin, A.; Daoud, S. The protective effect of a purified extract of *Withania somnifera* against doxorubicin-induced cardiac toxicity in rats. *Cell Biol. Toxicol.,* **2008**, *24*(1), 63-73. http://dx.doi.org/10.1007/s10565-007-9016-z PMID: 17520333

[27] Davis, L.; Kuttan, G. Immunomodulatory activity of *Withania somnifera. J. Ethnopharmacol.,* **2000**, *71*(1-2), 193-200. http://dx.doi.org/10.1016/S0378-8741(99)00206-8 PMID: 10904163

[28] Sankar, S.R.; Manivasagam, T.; Krishnamurti, A.; Ramanathan, M. The neuroprotective effect of *Withania somnifera* root extract in MPTP-intoxicated mice: an analysis of behavioral and biochemical variables. *Cell. Mol. Biol. Lett.,* **2007**, *12*(4), 473-481. http://dx.doi.org/10.2478/s11658-007-0015-0 PMID: 17415533

[29] Jayaprakasam, B.; Zhang, Y.; Seeram, N.P.; Nair, M.G. Growth inhibition of human tumor cell lines by withanolides from *Withania somnifera* leaves. *Life Sci.,* **2003**, *74*(1), 125-132. http://dx.doi.org/10.1016/j.lfs.2003.07.007 PMID: 14575818

[30] Bhattacharya, S.K.; Kumar, A.; Ghosal, S. Effects of glycowithanolides from *Withania Somnifera* on an animal model of Alzheimer's disease and perturbed central cholinergic markers of cognition in rats. *Phytother. Res.,* **1995**, *9*, 110-113. http://dx.doi.org/10.1002/ptr.2650090206

[31] Naidu, P.S.; Singh, A.; Kulkarni, S.K. Effect of *Withania somnifera* root extract on reserpine-induced orofacial dyskinesia and cognitive dysfunction. *Phytother. Res.,* **2006**, *20*(2), 140-146. http://dx.doi.org/10.1002/ptr.1823 PMID: 16444668

[32] Tong, X.; Zhang, H.; Timmermann, B.N. Chlorinated Withanolides from *Withania somnifera. Phytochem. Lett.,* **2011**, *4*(4), 411-414. http://dx.doi.org/10.1016/j.phytol.2011.04.016 PMID: 22125584

[33] Zhang, H.; Samadi, A.K.; Cohen, M.S.; Timmermann, B.N. Antiproliferative withanolides from the Solanaceae: a structure-activity study. *Pure Appl. Chem.,* **2012**, *84*(6), 1353-1367. http://dx.doi.org/10.1351/PAC-CON-11-10-08 PMID: 24098060

[34] Stan, S.D.; Hahm, E.R.; Warin, R.; Singh, S.V. Withaferin A causes FOXO3a- and Bim-dependent apoptosis and inhibits growth of human breast cancer cells *in vivo*. *Cancer Res.,* **2008**, *68*(18), 7661-7669. http://dx.doi.org/10.1158/0008-5472.CAN-08-1510 PMID: 18794155

[35] Thaiparambil, J.T.; Bender, L.; Ganesh, T.; Kline, E.; Patel, P.; Liu, Y.; Tighiouart, M.; Vertino, P.M.; Harvey, R.D.; Garcia, A.; Marcus, A.I. Withaferin A inhibits breast cancer invasion and metastasis at sub-cytotoxic doses by inducing vimentin disassembly and serine 56 phosphorylation. *Int. J. Cancer,* **2011**, *129*(11), 2744-2755. http://dx.doi.org/10.1002/ijc.25938 PMID: 21538350

[36] Hahm, E.R.; Lee, J.; Singh, S.V. Role of mitogen-activated protein kinases and Mcl-1 in apoptosis induction by withaferin A in human breast cancer cells. *Mol. Carcinog.,* **2014**, *53*(11), 907-916. http://dx.doi.org/10.1002/mc.22050 PMID: 24019090

[37] Hahm, E.R.; Lee, J.; Huang, Y.; Singh, S.V. Withaferin a suppresses estrogen receptor-α expression in human breast cancer cells. *Mol. Carcinog.,* **2011**, *50*(8), 614-624. http://dx.doi.org/10.1002/mc.20760 PMID: 21432907

[38] *Maestro (Version 9.6)*; Schrödinger, LLC: New York, NY, **2013**.

[39] *Accelrys Software Inc: Discovery studio Visualizer 4.0.,* **2014**.http://accelrys.com/products/discovery-studio/visualization-download.php

[40] Irwin, J.J.; Shoichet, B.K. ZINC--a free database of commercially available compounds for virtual screening. *J. Chem. Inf. Model.,* **2005**, *45*(1), 177-182. http://dx.doi.org/10.1021/ci049714+ PMID: 15667143

[41] Goodsell, D.S.; Morris, G.M.; Olson, A.J. Automated docking of flexible ligands: applications of AutoDock. *J. Mol. Recognit.,* **1996**, *9*(1), 1-5. http://dx.doi.org/10.1002/(SICI)1099-1352(199601)9:1<1::AID-JMR241>3.0.CO;2-6 PMID: 8723313

[42] *Shaw Research; Maestro-Desmond Interoperability Tools (Version 3.6)*; Schrödinger: New York, NY, **2013**.

[43] Berman, H.M.; Westbrook, J.; Feng, Z.; Gilliland, G.; Bhat, T.N.; Weissig, H.; Shindyalov, I.N.; Bourne, P.E. The protein data bank. *Nucleic Acids Res.,* **2000**, *28*(1), 235-242. http://dx.doi.org/10.1093/nar/28.1.235 PMID: 10592235

[44] Reddy, S.V.G.; Reddy, K.T.; Kumari, V.V.; Basha, S.H. Molecular docking and dynamic simulation studies evidenced plausible immunotherapeutic anticancer property by Withaferin A targeting indoleamine 2,3-dioxygenase. *J. Biomol. Struct. Dyn.,* **2015**, *33*(12), 2695-2709. http://dx.doi.org/10.1080/07391102.2015.1004834 PMID: 25671592

[45] Basha, S.H.; Bethapudi, P.; Majji Rambabu, F. Anti-angiogenesis property by Quercetin compound targeting VEGFR2 elucidated in a computational approach. *Eur. J. Biotech. Biosci.,* **2014**, *2*, 30-46.

[46] Basha, S.H.; Kumar, K.N. Ligand and Structure based virtual screening studies to identify potent inhibitors against herpes virus targeting gB-gH-gL complex interface as a novel drug target. *Open Access Sci Rep.,* **2012**, *1*, 566.

[47] Rao, C.M.; Yejella, R.P.; Rehman, R.S.A.; Basha, S.H. Molecular docking based screening of novel designed chalcone series of compounds for their anti-cancer activity targeting EGFR kinase domain. *Bioinformation,* **2015**, *11*(7), 322-329. http://dx.doi.org/10.6026/97320630011322 PMID: 26339147

[48] Murthy, N.V.; Girija Sastry, M.V.; Basha, S.H. 3, 5-dinitrophenyl clubbed azoles against latent tuberculosis-a theoretical mechanistic study. *J. PeerSci.,* **2018**, *1*(1)e1000001

[49] Tripathy, S.; Sahu, S.K. In-silico studies on Molecular Orbital's, Geometry Optimization and Molecular Docking of Curcumin as an antibacterial drug targets FtsZ protein. *J. Peer Sci.,* **2018**, *1*(2)e1000006.