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*Research Article*

# ANTINEOPLASTIC EFFICACY OF *SMILAX WIGHTII* A.DC. (SMILACACEAE) AND ITS STEROIDAL SAPOGENINS: *IN VITRO* TO *IN SILICO* APPROACHES

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**ABSTRACT.** Competency of plant metabolites and derivatives as antineoplastic drugs has been validated by modern medicine and the screening of flora for anticancer agents is crucial in drug discovery. *Smilax wightii* A.DC. (Smilacaceae) is an ethnomedicinal plant endemic to Western Ghats of India. The methanolic extract of various plant parts were subjected to investigation of antiproliferative potential. Dalton's lymphoma Ascites (DLA), Ehrlich-Lettre ascites carcinoma (EAC), rat spleen, mouse fibroblast (L929) and human lung cancer (A549) cell lines were employed in the analysis. Stem extract exhibited high inhibitory property at 200 µg/mL on both DLA and EAC cell lines with 92.43±0.176 and 90.42±0.272% cell death respectively. In the spleen cell line root extract recorded the lowest cytotoxicity. The leaf and root extracts were subjected to MTT assay in human lung cancer (A549) and mouse Fibroblast (L929) cell lines to determine their anticancer property. Root recorded better apoptotic effect on the mouse fibroblast cell line and lung cancer cell line when compared to leaf extract. Molecular docking of the compounds Sarsasapogenin and Diosgenin present in in the plant to potential anticancer target proteins *viz* HSP-90 alpha and LXR-alpha verified their anticancer potential. Sarsasapogenin could bind to the active site of HSP-90 alpha with the low binding energy of -9.33Kcal/Mol and Diosgenin interacted with the active site of LXR-alpha with low binding energy of -6.52Kcal/Mol. These molecules are among the many bioactive principles that are ground to the anticancer activity of the crude extract and are eligible to be prospective antineoplastic drugs.

**Keywords:** *Smilax wightii, cytotoxicity, sarsasapogenin, diosgenin, HSP-90 alpha, LXR-alpha*

## INTRODUCTION

*Smilax wightii* is a prickly woody climber in the family Smilacaceae, rare and endemic to the Western Ghats. According to the literature, Ayurveda used its root to cure diseases of the nervous system and urinary system. In other Indian and Chinese systems of medicine, the plant is applied to cure skin diseases, sores, swelling, abscesses, gastric complaints, venereal diseases [1]. The plant's medicinal properties include anti-inflammatory, antioxidant and antidiabetic activities were reported earlier [2,3,4]. The anticancer property of the plant needs to be evaluated, which will fuel the investigation for novel antineoplastic agents.

The bioactive metabolites in plants have diverse therapeutic potential along with proven effect on mammalian cells. Oncogenesis is the uncontrolled cell division in the body leading to the development of tumours. This is a a major health hazard in the world that costs lives and a major share of the economy. The commonly employed chemotherapy is associated with many short and long term after effects that calls for natural compound

based therapy to combat cancer. Plants have proved to be reliable sources of compounds that can inhibit the progression of malignancies. Investigation of plants for potent molecular structures to be used in the treatment of tumors is a principal research objective in the world. Scientists have successfully isolated many anticancer agents from floras and numerous such compounds are on clinical trials.

*In vitro* cytotoxicity assays using cell lines are reliable and rapid methods for analyzing chemotherapeutic activity of compounds [5]. This method is being widely used to evaluate the effect of molecules on tumor progression. *In silico* studies will also help to elucidate the properties of bioactive molecules and their interactions with the biomolecules within the system. The specific objectives of the study include (1) Evaluation of the antiproliferative potential of various parts of *S. wightii* using *in vitro* cell line based assays (2) To conduct *in silico* studies on the compounds Sarsasapogenin and Diosgenin. (3) Molecular docking studies on these molecules with selected anticancer target proteins

## MATERIALS AND METHODS

### *Plant collection and solvent extraction*

Plant materials were collected from Idinjar forest range of Thiruvananthapuram District, Kerala, India at 8.751855°N and 77.070835°S. A specimen of the plant was submitted at the herbarium of the Department of Botany, University of Kerala (voucher no: KUBH 10531). Leaf, stem, rhizome and root of *S. wightii* were separated and washed in running water. It was then shade dried, powdered and stored in an airtight container. The powders were subjected to hot continuous extraction in a Soxhlet apparatus for 6 hours, serially using solvents of increasing polarity viz Petroleum ether, Chloroform, Methanol and Distilled water. The collected extract was concentrated in a rotary evaporator and stored in a refrigerator for further analysis.

### *Evaluation of Anticancer Activity*

Trypan blue exclusion method and MTT assay were performed on cancer and normal cell lines to determine the anticancer potential of various plant parts of *S. wightii.*

### *Trypan blue exclusion assay*

A viable cell suspension ($1\times10^6$ cells in 0.1ml) of DLA and EAC cell line from mice and rat spleen cells were added to tubes containing various concentrations of the plant extract. They were made up to 1 ml using PBS in case of DLA and EAC cells and RPMI complete medium in the rat spleen tissue. The control tube contained only cell suspension in the three cases. These assay mixtures were incubated at 37° C for 3 hours. Later the cell suspension was treated with 0.1 ml of 1% trypan blue and loaded on a hemocytometer after 2-3 minutes incubation. The stained and unstained cells were counted separately and cytotoxicity was calculated using the equation:

$$\text{Percentage of cytotoxicity} = \frac{\text{Number of dead cells}}{\text{Number of live cells+Number of dead cells}} \times 100$$

### *MTT assay*

MTT assay was conducted on the L929, and A549 cell lines [6]. These cell lines were procured from National Centre for Cell Sciences (NCCS), Pune, India and maintained in Dulbecos modified Eagles medium (Gibco, Invitrogen). They were cultured in 25 $cm^2$ tissue culture flask containing DMEM supplemented with 10% FBS, L-glutamine, sodium bicarbonate and antibiotic solution containing: Penicillin (100U/ml), Streptomycin (100µg/ml) along with Amphoteracin B (2.5µg/ml) and incubated at 37ºC in a humidified 5% $CO_2$ incubator (NBS Eppendorf, Germany). After 4 hours, cells were trypsinized and suspended in 10% growth medium. 100µl cell suspension ($5x10^4$ cells/well) was seeded in 96 well tissue culture plates and incubated in a 5% $CO_2$ incubator for 24 hours. 100µl of the samples prepared in 5% DMEM was added to the wells after removing the growth medium and incubated in a 5% $CO_2$ incubator. Samples were removed from wells after 24 hours and added 30µl of MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide) reconstituted in PBS. It was shaken gently and incubated for 4 hours. After removing the supernatant, 100µl of DMSO was added to solubilize the formazan crystals. Absorbance was recorded at 540 nm using a microplate reader. The percentage of cell viability was calculated using the formula:

$$\text{Percentage of viability} = \frac{\text{Mean OD Samples}}{\text{Mean OD of control group}} \times 100$$

### *Statistical analysis*

The experiments were conducted in triplicates and the data were analysed through one-way analysis of variance using SPSS software (ver. 22.0; SPSS Inc., Chicago, IL, USA). The results were represented as mean ± SE (Standard error) of the experiments. The values are significant at $P<0.05$ level.

### *Molecular Docking*

The SMILES (simplified molecular input line entry system) notation of the compounds Sarsasapogenin and Diosgenin were obtained from Pubchem (https://pubchem.ncbi.nlm.nih.gov/) and the three-dimensional structure was created with the help of Corina Demo tool (https://www.mn-am.com/online_demos/corina_demo). The structures were visualized in Pymol, ADME properties (Absorption, Distribution, Metabolism, and excretion) studied using the web tool SwissADME (http://www.swissadme.ch/) and the high probability targets of the molecules were predicted using Swiss target prediction (http://www.swisstargetprediction.ch/). The structures of the target proteins were obtained from Protein Data Bank (https://www.rcsb.org/) and Molecular Docking studies were conducted with the help of AutoDock 4.2 software. The docked conformation of the ligand – protein complex was visualized using the software Discovery Studio visualizer.

## RESULTS AND DISCUSSION

### *Evaluation of Anticancer Activity*

Petroleum ether, chloroform, methanol and aqueous extracts of the four plant parts of *S. wightii* were subjected to preliminary phytochemical screening using standard

procedures, revealing the highest number of phytoconstituents in the methanolic extract of all the plant parts when compared to the extraction using the low polarity solvents. So the methanolic extract of Leaf (LME), stem (SME), rhizome (RHME) and root (RME) of *S. wightii* were selected for the present investigation. Cell-based assays could be effectively used to analyze the susceptibility of various target cell lines to chemotherapeutic agents [7]. The anticancer activity of all the parts of *S. wightii* was analyzed using five cell lines.

***Trypan blue exclusion assay***

In the assay to determine the cytotoxicity, dead cells take up the blue colour of trypan blue while live cells do not take up the dye. Dalton's lymphoma Ascites cell line is T-*cell lymphoma* from the thymus of murine host. The experimental results were statistically analysed through one-way ANOVA. The data were represented as mean ± Standard error, where f values are significant at $P<0.05$ level.SME showed comparatively high inhibition to the multiplication of DLA cells followed by RME, RHME and LME respectively (Table 1). All the extracts showed a concentration-dependent increase in the percentage of cell death. Stem extract exhibited 92.43±0.176% cell death at 200 µg/mL. Ehrlich-Lettre ascites carcinoma is the ascites tumor cell line from mice. LME, SME, RHME, and RME exhibited concentration-dependent increase in cytotoxicity (Table 2). SME recorded the highest activity of 90.42±0.272% cell death at 200 µg/mL. RME also exhibited considerable activity in both cell lines.

The influence of the plant extracts on the normal cells needs to be evaluated in order to predict the possibility of recommending the plant extract for therapeutic purposes. The extract that reduces the viability of the normal cell line could be toxic to health and can't be recommended as alleviative. All the samples showed reduced inhibitory property in the rat spleen cell line (Table 3). LME, SME and RME showed no cytotoxicity in the concentrations ranging from 10-100 µg/mL. At 200µg/mL, LME, SME and RME exhibited mild cytotoxicity of 5.067±0.05, 4.033±0.115 and 2.033±0.208%, respectively. Comparatively enhanced cytotoxicity was recorded by RHME. At 100 and 200 µg/mL it exhibited cytotoxicity of 3.0±0.10 to 8.067±0.251%. Lowest cytotoxicity was recorded by RME. Here all the samples reduced the viability of DLA and EAC cell lines and showed least toxicity on rat spleen cell lines. Increased toxicity on the cancer cell lines and reduced toxicity on the normal cell lines is a favorable property for antineoplastic agents.

***Table 1.*** *Cytotoxicity of S.wightii extracts on DLA cell line*

| **Sample Concentration (µg/mL)** | **Percentage of cell death in Dalton's lymphoma Ascites cells (DLA)** | | | |
|---|---|---|---|---|
| | **LME** | **SME** | **RHME** | **RME** |
| 10 | 7.63±0.26 | 68.6±0.208 | 47.66±0.176 | 28.5±0.115 |
| 20 | 18.06±0.145 | 71.4±0.0.264 | 55.3±0.208 | 42.86±0.233 |
| 50 | 35.9±0.251 | 78.53±0.233 | 61.9±0.173 | 52.3±0.264 |
| 100 | 53.36±0.24 | 84.36±0.145 | 76.1±0.23 | 68.63±0.145 |
| 200 | 74.26±0.24 | 92.43±0.176 | 83.86±0.233 | 85.73±0.176 |

The representative experiment is a mean standard error, f value significant at $P<0.05$ level. The control tube had only one dead cell.

***Table 2.*** *Cytotoxicity of S.wightii extracts on EAC cell line*

| Sample Concentration (µg/mL) | Percentage of cell death in Ehrlich-Lettre ascites carcinoma (EAC) | | | |
|---|---|---|---|---|
| | **LME** | **SME** | **RHME** | **RME** |
| 10 | 13.3±0.264 | 66.6±0.152 | 47.26±0.233 | 30.4±0.251 |
| 20 | 23.8±0.115 | 72.33±0.202 | 52.76±0.176 | 43.66±0.145 |
| 50 | 43.53±0.233 | 78.0±0.23 | 59.16±0.233 | 50.4±0.152 |
| 100 | 64.66±0.29 | 82.8±0.173 | 74.36±0.145 | 67.26±0.26 |
| 200 | 78.06±0.24 | 90.43±0.272 | 81.96±0.24 | 85.867±0.176 |

The representative experiment is a mean standard error, f value significant at P<0.05 level. The control tube had only one dead cell.

***Table 3.*** *Cytotoxicity of S.wightii extracts on Rat spleen cell line*

| Sample Concentration(µg/mL) | Percentage of cell death in rat spleen cell line | | | |
|---|---|---|---|---|
| | **LME** | **SME** | **RHME** | **RME** |
| control | 0.0±0.0 | 0.0±0.0 | 0.0±0.0 | 0.0±0.0 |
| 10 | 0.0±0.0 | 0.0±0.0 | 0.0±0.0 | 0.0±0.0 |
| 20 | 0.0±0.0 | 0.0±0.0 | 0.0±0.0 | 0.0±0.0 |
| 50 | 0.0±0.0 | 0.0±0.0 | 0.0±0.0 | 0.0±0.0 |
| 100 | 0.0±0.0 | 0.0±0.0 | 3.0±0.10 | 0.0±0.0 |
| 200 | 5.067±0.057 | 4.033±0.115 | 8.067±0.251 | 2.033±0.208 |

The representative experiment is a mean standard error, f value significant at P<0.05 level

***MTT assay***

MTT is a colorimetric assay that depends on the cellular reduction of yellow-colored tetrazolium salts to purple-colored formazan crystals by cellular enzymes in metabolically active cells. This widely accepted and reliable method for determining the cytotoxicity of samples was conducted with LME and RME on mouse fibroblast (L929) and human lung cancer (A549) cell lines. Statistical analysis of the experimental data was done through one-way ANOVA. The results were represented as mean ± Standard error, where f values are significant at P<0.05 level. Both extracts showed a dose-dependent increase in the antiproliferative activity on the cell lines along with the induction of apoptosis (Table 4 and 5). On the A549 cell line RME had better inhibitory activity with low IC50 value of 58.913±3.483 µg/mL, whereas LME exhibited higher IC50 (111.95±0.357µg/mL). On the L929 cell line also IC50 concentration of RME (135.523±0.949 µg/mL) was recorded to be lower than that of LME. So RME had a better apoptotic effect on the mouse fibroblast cell line and lung cancer cell line when compared to LME. In the experiment, 5FU was used as the standard in L929 cell line and Doxorubicin was used as the standard drug in A549 cell line.

From the MTT and trypan blue exclusion assays, it was evident that the extracts of *S. wightii* could inhibit cell proliferation leading to oncogenesis. So there is some bioactive principle in the plant with anticancer property. Isolation and identification of the

molecules responsible for its antineoplastic potential can boost the drug development programs. Polyphenolic compounds like flavonoids, tannins, terpenoids, alkaloids and brassinosteroids are reported cytotoxic agents in plants. Modern research is enquiringly focused on identifying and isolating the active principle in medicinal plants. There are many plant derived antineoplastic drugs in modern medicine. Podophyllotoxin, isolated from *Podophyllum peltatum*, Vincristine and Vinblastine from *Catharanthus roseus*, Camptothecin isolated from *Camptotheca acuminate*, Taxol isolated from *Taxus brevifolia* and Homoharringtonine isolated from *Cephalotaxus hainanensis* are few prominent anticancer compounds having plant origin [8,9,10,11,12].

***Table 4.*** *Antiproliferative activiy of S .wightii extracts on L929 cell line*

| **Sample Concentration (µg/mL)** | **Percentage of cell viability in L929 cell line** | | |
|---|---|---|---|
| | **LME** | **RME** | **5FU** |
| control | 100±0.00 | 100±0.00 | 100±0.00 |
| 6.25 | 94.18±0.078 | 96.32±0.067 | 92.45±0.02 |
| 12.5 | 72.83±0.011 | 92.94±0.132 | 79.71±0.030 |
| 25.0 | 70.27±0.138 | 92.47±0.126 | 75.29±0.036 |
| 50.0 | 63.28±0.087 | 81.32±0.22 | 66.71±0.026 |
| 100.0 | 53.27±0.074 | 78.32±0.156 | 51.39±0.017 |

The representative experiment is a mean standard error, f value significant at P<0.05 level.

***Table 5.*** *Antiproliferative activity of S. wightii extracts on A549 cell line*

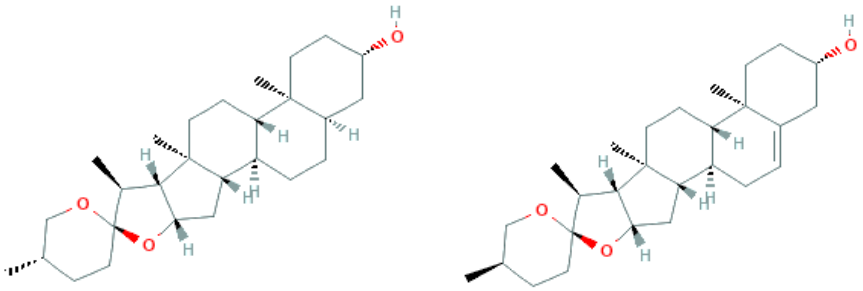
| **Sample Concentration(µg/mL)** | **Percentage of cell viability in A549 cell line** | | |
|---|---|---|---|
| | **LME** | **RME** | **Doxorubicin** |
| control | 100±0.00 | 100±0.00 | 100±0.00 |
| 6.25 | 71.22±0.02 | 64.47±0.131 | 65.31±0.015 |
| 12.5 | 62.91±0.067 | 60.58±0.173 | 52.27±0.065 |
| 25.0 | 61.506±0.089 | 59.77±0.135 | 43.37±0.040 |
| 50.0 | 55.386±0.079 | 59.26±0.236 | 32.46±0.045 |
| 100.0 | 56.53±0.095 | 52.55±0.127 | 20.78±0.040 |

The representative experiment is a mean standard error, f value significant at P<0.05 level.

***Molecular Docking***

Sarsasapogenin and Diosgenin are two steroidal sapogenins reported in the genus *Smilax* and High-performance thin-layer chromatographic studies confirmed their presence in LME of *S. wightii. In silico* studies could be effectively used to predict the drug-likeness of the molecules of interest before initiating wet-lab experiments. Through ADME property prediction and target prediction, we can foresee the behavior of the molecule once it enters the biological system as a drug molecule and identifies the target proteins it can interact to within the system. Molecular docking helps to visualize and analyze the different conformations of the ligand molecule in the binding site of the target

protein. The high-affinity binding of the ligand to the binding pocket of the target protein with the lowest binding energy is the favorable interaction.



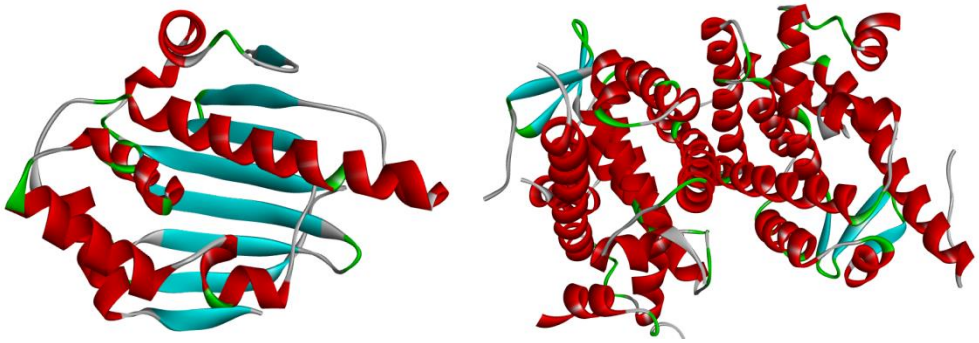
(a) (b)

***Fig. 1.*** *Structure of (a) Sarsasapogenin (b) Diosgenin* (https://pubchem.ncbi.nlm.nih.gov)

Sarsasapogenin reported from the plant has favorable ADME properties (Fig. 1a and Table 6). The molecule violate one parameter in Lpinski's rule of five for orally bioavailable drugs, in having the Consensus Log $P_{o/w}$ value 5.21 (>5). Through Swiss target prediction Heat shock protein HSP 90-alpha was observed to be its highest probability target. This is a chaperone protein involved in many cellular functions along with the maturation and regulation of target proteins involved in cell cycle control and signal transduction and stabilizes a number of proteins required for tumor growth. The inhibition of HSP 90-alpha will lead to proteasomal degradation of the client proteins that results in antitumor activity [13]. Also, Heat-shock proteins are observed in increased levels in several tumors and haematological malignancies [14]. So inhibitors of Hsp90 could be explored as potential anticancer agents. [1] The X-ray Diffraction structure of Heat Shock Protein 90 Bound to CS301 with a resolution of 1.91 Å was retrieved from Protein data bank and modified for docking studies (Fig. 2a). The Ramachandran Plot representing the torsional angles of the residues contained in HSP 90-alpha indicated the presence of more β sheets and α helix along with some left handed helix in the peptide (Fig. 3a). Docked conformation of the ligand – protein complex was visualized using Discovery Studio visualizer (Fig. 4a, 4b). The binding energy was -9.33Kcal/Mol. The analysis proved that Sarsasapogenin could be an eligible ligand in antineoplastic drug discovery and has the potential to be developed into a drug that targets HSP 90-alpha. There are nearly 17 distinct HSP 90 inhibitors going through clinical trials for cancer therapy [15]. So scientists have recognized the potential of this target protein in drug discovery.

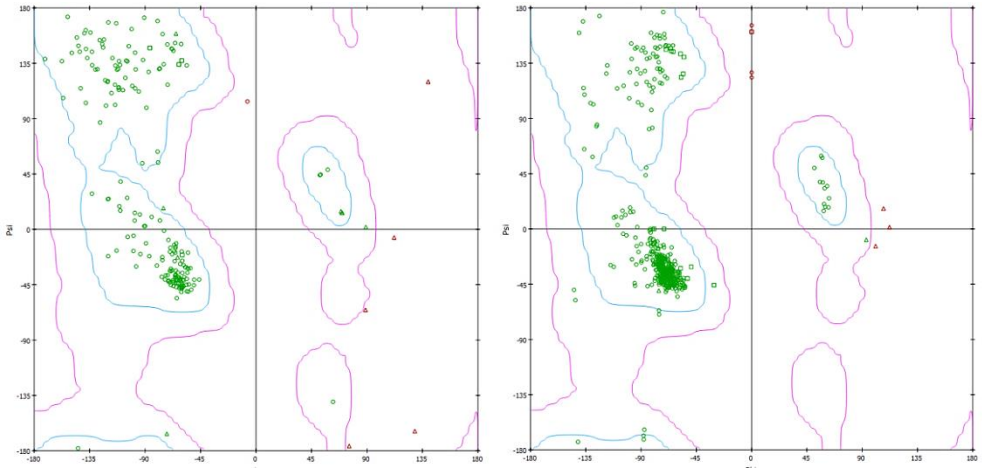
***Table 6.*** *ADME properties of Sarsasapogenin and Diogenin*

| **Property** | **Sarsasapogenin** | **Diosgenin** |
|---|---|---|
| Formula | $C_{27}H_{44}O_3$ | $C_{27}H_{42}O_3$ |
| Molecular weight | 416.64 g/mol | 414.62 g/mol |
| Num. H-bond acceptors | 3 | 3 |
| Num. H-bond donors | 1 | 1 |
| Molar Refractivity | 122.07 | 121.59 |
| TPSA | 38.69 Å² | 38.69 Å² |
| Consensus Log $P_{o/w}$ | 5.21 | 5.00 |
| Log $K_p$ (skin permeation) | -4.23 cm/s | -4.80 cm/s |
| Bioavailability Score | 0.55 | 0.55 |
| Synthetic accessibility | 6.88 | 6.94 |



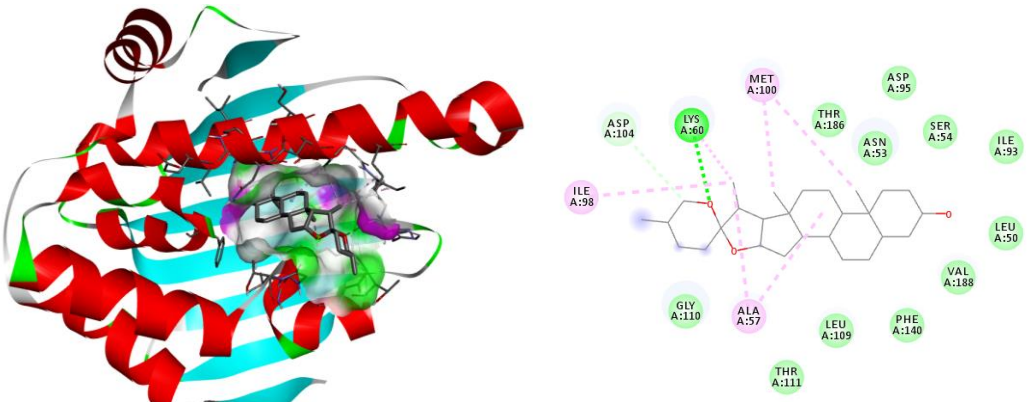
(a) (b)

***Fig. 2.*** *Structure of (a)HSP 90-alpha (b) LXR-alpha (*https://www.rcsb.org/)



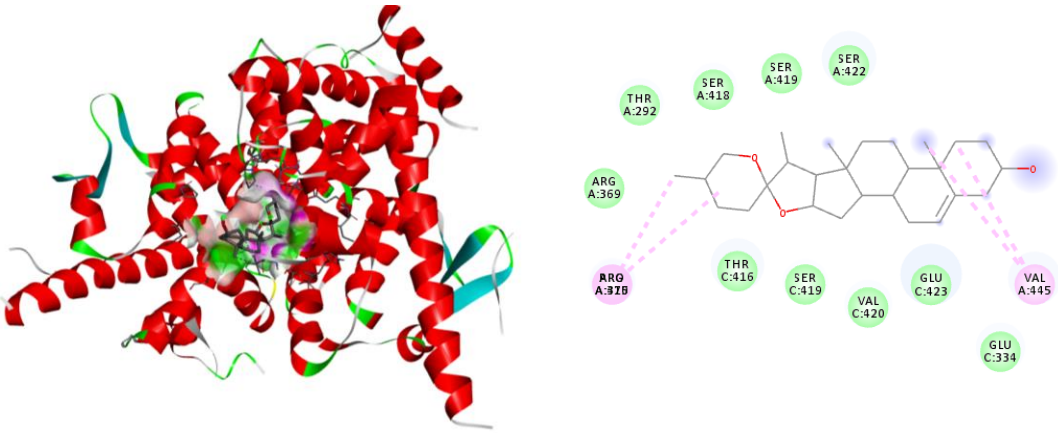
(a) (b)

***Fig. 3.*** *Ramachandran plot of (a)HSP 90-alpha and (b) LXR-alpha captured using Discovery studio visualizer software*




(a) (b)

***Fig. 4.*** *Sarsasapogenin in the binding pocket of HSP 90-alpha (a)2D Diagram of Sarsasapogenin- HSP 90-alpha interactions, captured using Discovery studio visualizer software*




(a) (b)

***Fig. 5.*** *Diosgenin in the binding pocket of LXR-alpha (a)2D Diagram of Diosgenin - LXR-alpha interactions, captured using Discovery studio visualizer software*

Diosgenin is another steroidal sapogenin in *Smilax* species that indicated appreciable drug-likeness properties in SwissADME property prediction (Fig. 1b and Table 6). It also obeyed the Lpinski's rule of five for orally bioavailable drugs, and has the Consensus Log $P_{o/w}$ value 5. LXR-alpha protein was predicted to be the highest probability target of Diosgenin. This is a protein encoded by NR1H3 gene in the nuclear receptor superfamily which includes the DNA-binding btranscription factors involved in the control of cholesterol, glucose and fatty acid. The LXR isoform LXR-alpha functions mostly in the liver, kidney, intestine, fat tissue, macrophages, lung, and spleen [16]. There are reports regarding the synthetic and natural LXR agonists that can inhibit the *in vitro* proliferation of prostate and breast cancer cells [17,18,19]. Studies have proved the function of LXRs in many cancers and their potential as prospective targets for anticancer therapy [20]. Ju et al., [21] reviewed the action of LXRs in various cancer and their applications in clinical

treatment. There are reports regarding LXR inhibiting estrogen-dependent cancer cell proliferation by regulating the hepatic expression of Estrogen sulfotransferase in breast cancer models [22]. LXR-alpha signaling in cells of chronic lymphocytic leukemia patients is being studied to analyse the possible modulation of LXR-alpha as a potential therapeutic target in the treatment of chronic lymphocytic leukemia [23]. LXR agonists also have antitumour potential. The X-ray Diffraction structure of LXR-alpha in complex with tert-butyl benzoate analog, compound 4, with a resolution of 2.7 Å submitted by Matsui et al., in Protein data bank was modified for molecular docking (Fig. 2b) [24]. The Ramachandran Plot representing the torsional angles of the residues contained in HSP 90-alpha indicated the presence of β sheets, α helix as well as left handed helix in the peptide (Fig. 3b). The binding energy was recorded to be-6.52Kcal/Mol. The docking studies pointed to the fact that the compound Diosgenin has the potential to bind to the active site of LXR-alpha and modify its activity (Fig. 5a, 5b). From the literature, it is clear that the potential of the ligands like Sarsasapogenin and Diosgenin to modify the action of cancer related protein could be used efficiently in drug discovery for suppression of oncogenesis. The presence of Sarsasapogenin and Diosgenin could be a reason for the anticancer activity exhibited by the crude methanol extracts of various parts of *S. wightii.* But there is a possibility for the presence of other novel bioactive molecules as well in the plant, which accounts for its curative efficacy.

## CONCLUSION

*Smilax wightii* A.DC. is a rare and endemic plant in the Western Ghats with ethnomedicinal importance. Methanolic extract of the leaf, stem, rhizome, and root were evaluated for their anticancer property. Trypan blue exclusion method and MTT assay on DLA, EAC, rat Spleen, L929 and A549 cell lines were used to study the antiproliferative activity of the plant parts. Stem extract exhibited high cytotoxicity on DLA and EAC cell lines at 200 µg/mL followed by the root, rhizome and leaf respectively. On the Spleen cell line, all the extracts showed the least cytotoxicity. In the MTT assay, root extract exhibited better apoptotic effect on the mouse fibroblast cell line (L929) and lung cancer cell line (A549) when compared to leaf. The analysis proved that there is some phytoconstituent in *S. wightii* that can inhibit tumor progression. The presence of Sarsasapogenin and Diosgenin in LME was confirmed by chromatographic studies. Molecular docking studies with potential anticancer targets viz HSP 90-alpha and LXR-alpha confirmed that these molecules can modify the function of these target proteins and could be used in the pharmaceutical industry against cancer.

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**Conflict of interest.** The authors declared that there is no conflict of interest

**Authorship contributions.** Concept: S.T.S, A.VA., Design: S.T.S, A.VA., Data Collection or Processing: S.T.S., A.VA., Analysis or Interpretation: S.T.S., A.V.A, Literature Search: A.V.A, Writing: S.T.S., A.V.A.

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