

Research article

Phytocompounds from *Withania somnifera* against breast cancer: An *in-silico* study

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(Received: November 2021

Revised: July 2022

Accepted: July 2022)

Corresponding author: **Kanagaraj Jyothi**. Email: drjyothisamy@gmail.com**ABSTRACT**

Introduction and Aim: *Withania somnifera*, called as Indian Ginseng is a very important plant in traditional medicinal practices. The plant possesses a wide range of activity and has been used to treat multiple diseases. Ginseng possesses anti-inflammatory, neuroprotective, hypoglycemic, antiarthritic, hepatoprotective, antioxidant, anti-stress, immunostimulatory and anti-cancer therapeutic activities. Cancer is a health burden prevalent worldwide and, breast cancer is the top major cause of death among women and people in the low and middle-income countries are affected in higher number because they have low treatment access. This is an *in-silico* study and focused on studying the interaction between five phytocompounds namely, anaferine, isopelletierin, sitoindoside IX, somniferine, withanone present in *W.somnifera* and the 3 proteins involved in breast cancer pathway viz., C-Raf, Akt 2 and GSK 3 β through molecular docking.

Methodology: We retrieved the above three proteins from PDB, retrieved five ligands from PubChem, and docking was done. Docking of the phytocompounds against the target proteins were carried out using Auto dock vina.

Results: From the docking results, we found that the phytocompounds; sitoindoside IX, somniferine, withanone from *Withania somnifera* are effective in inhibiting the proteins causing breast cancer whereas anaferine and isopelletierin are less effective in inhibiting the breast cancer.

Conclusion: This study concludes that the phytocompounds sitoindoside IX, somniferine, withanone from *W. somnifera* have the potential ability to treat breast cancer. These findings will aid in the development of natural based therapy against breast cancer.

Keywords: *Withania somnifera*, Molecular docking; C-Raf; AKt 2; GSK 3 β .

INTRODUCTION

Cancer is one of the major health burdens worldwide. Breast cancer accounts for nearly 30% of all cancer and one of the dominant causes of death among women. Cancer progression is a cumulative effect of alteration in various cell signaling pathways. Of the many proteins involved in signaling, the proteins which have a crucial role in breast cancer were selected and used in the current study. The target receptors are C-Raf, GSK 3 β and Akt.

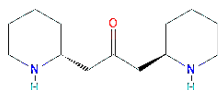
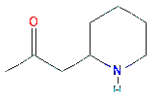
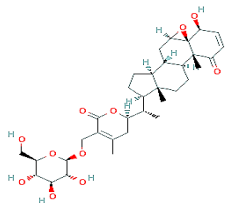
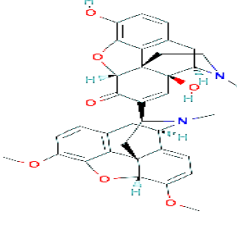
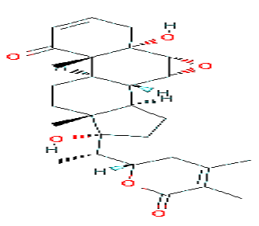
GSK-3 (Glycogen Synthase Kinase-3) is a serine/threonine protein kinase. In mammals, GSK-3 exists in two highly homologous forms; GSK-3 α and GSK-3 β . GSK-3 β acts as a positive control of cancer cell proliferation and survival. Overexpression of GSK-3 β in human breast carcinoma was reported. The risk of poor prognosis and relapse was also reported to be high. It was also reported that breast cancer cell proliferation can significantly inhibited by knockdown of GSK-3 β . (1).

Akt2 is a serine/threonine kinase that involved in the PI3K/AKT/mTOR signaling pathway and plays a major role in cell proliferation and survival. The role of Akt 2 in invasion and cancer metastases in already reported. In an intracellular signaling event, Akt is

recruited by the activity of PI3K in converting PIP2 to PIP3 (2). Akt is activated by phosphorylation, and it further phosphorylates and activate its targets, TSC2 (tuberos sclerosis complex 2), GSK 3 β , and FOXO (forkhead kinase transcription factors) that promotes cell proliferation and survival. Akt activation is reported to be a common event in human breast cancer (3,4).

Withania somnifera (Ashwagandha) called Indian ginseng belongs to the Solanaceae family. It is an annual green shrub that is highly prevalent in India (5). It is of prime importance in the traditional ayurveda medicinal practices on account of its varied pharmacological properties. The properties include anti-inflammatory, anti-tumor, anti-cancer, antioxidant, anti-stress, immune modulatory, anti-diabetic, cardio-protective, neuro-protective, and anti-microbial properties. The species name, *somnifera*, derived from Latin means 'sleep inducer'. *W. somnifera* exhibits all these properties due to the presence of various phytochemicals. The plant possesses alkaloids, flavonoids, steroids, steroidal lactones, and salts. All these components form the basis for the rich phyto-chemistry of the plant and hence its medicinal importance (6, 7).

Table 1: Phytocompounds selected from *Withania somnifera*

Compound name	PubChem ID	Type	2D - Structure
Anaferine	443143	Salt	
Isopelletierin	92987	Steroidal alkaloid	
Sitoindoside IX	189586	Steroid	
Somniferine	14106343	Alkaloid	
Withanone	21679027	Steroidal lactone	

In the present study Anaferine, Isopelletierin, Sitoindoside IX, Somniferine and Withanone are taken for molecular docking. The type and structure of the phytocompounds is given in Table 1. The screening and structural elucidation has exhilarated the potential of phytocompounds as chemotherapeutic agents. To develop any natural plant based products for therapeutic applications, need to understand and identify its targets and the mechanism of action (8). Molecular docking is the one which is extensively used in drug discovery to analyze the molecular behavior on target proteins (9).

MATERIALS AND METHODS

Identification and retrieval of proteins

The three-dimensional structures of the three proteins which involved in the progression of breast-cancer, namely, C-Raf (PDB ID: 3OMV), GSK 3 β (PDB ID: 6H0U) and Akt 2 (PDB ID: 3D0E) were identified through literature search and these protein structures were retrieved from RCSB PDB (<https://www.rcsb.org/>).

Identification and retrieval of phytocompounds

Phytocompounds from *W. somnifera* which could have potential as anti-cancer drugs, were identified from previously reported literatures (10). The 2D and 3D

structures of the phytocompounds were retrieved from PubChem. PubChem ID of the compounds are presented in Table 1. The compounds chosen are Anaferine, Isopelletierin, Sitoindoside IX, Somniferine and Withanone.

Preparation of proteins and docking

By using Auto Dock Tools, the proteins were prepared by (a) removing water molecules, (b) adding hydrogen molecules, (c) adding Kollman Charges. Docking of the phytocompounds against the target proteins were performed using Auto dock vina. Discovery studio was used for visualizing the protein-ligand interaction in 3D and 2D conformations.

RESULTS

The chosen phytocompounds name, Pub Chem IDs, types, 2D structures were presented in Table 1. The molecular docking of phytocompounds present in *W. somnifera* was carried out against proteins associated with breast cancer and the protein names, PDB ID, No. of residues/ nucleotides, ligands, docking scores are presented in Table 2. Somniferine showed the best interaction with C-Raf and Akt 2 proteins with binding energy of -9.0 Kcal/mol and -10.7 Kcal/mol respectively (Table 2). The 3D and 2D interactions are depicted in Fig.1 and Fig.2.

Table 2: Docking of Anaferine, Isopelletierine, Sitoindoside IX, Somniferine and Withanone to C-Raf, Akt 2, and GSK 3 β protein molecules

Protein	PDB ID	No. of residues/ nucleotides	Ligand	Binding energy (Kcal/mol)
C-Raf	3OMV	307	Anaferine	-6.1
			Isopelletierin	-5.7
			Sitoindoside IX	-8.7
			Somniferine	-9.0
			Withanone	-8.8
Akt 2	3D0E	335	Anaferine	-6.8
			Isopelletierin	-5.0
			Sitoindoside IX	-9.8
			Somniferine	-10.7
			Withanone	-9.5
GSK 3 β	6H0U	420	Anaferine	-6.1
			Isopelletierin	-4.7
			Sitoindoside IX	-10.1
			Somniferine	-8.7
			Withanone	-9.7

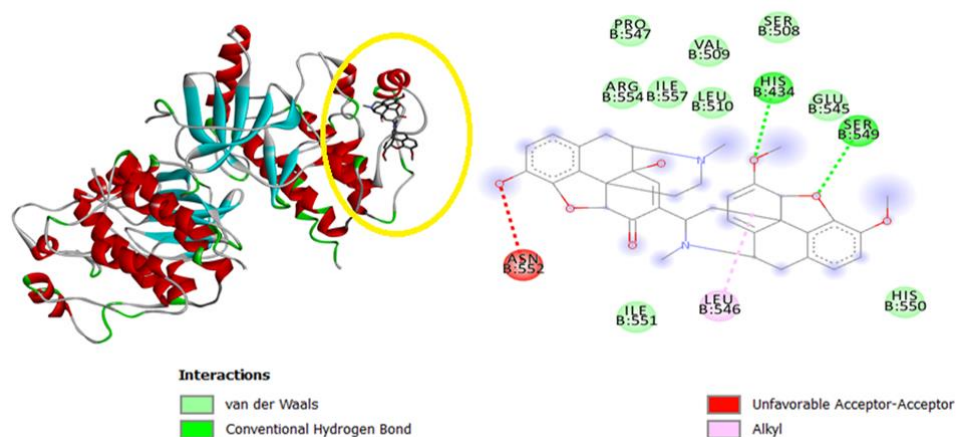


Fig. 1: 3D and 2D interaction of the target protein C-Raf with Somniferine

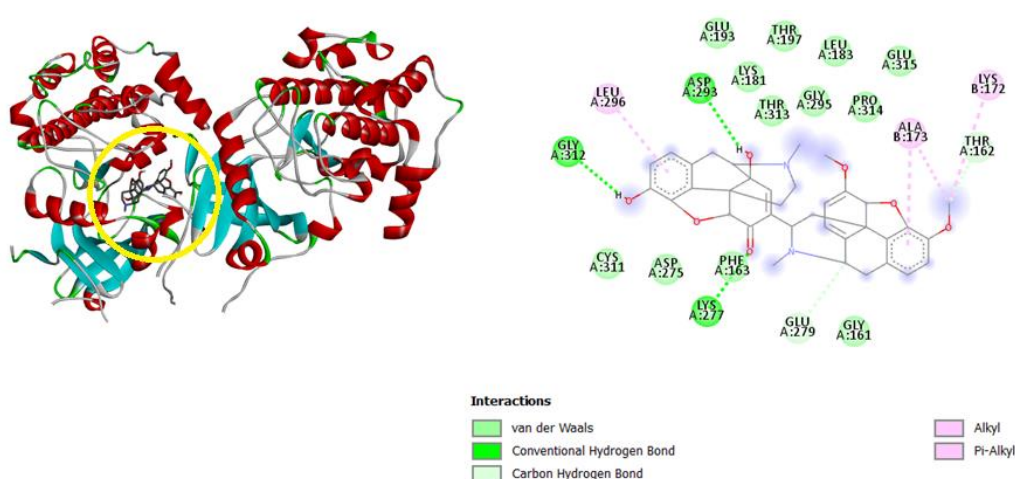


Fig. 2: The 3D and 2D interaction of the target protein Akt2 and the phytocompound Somniferine

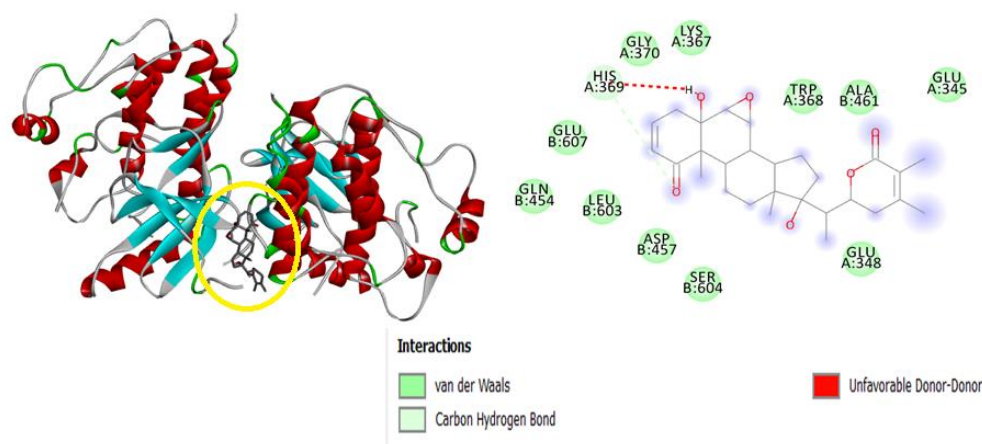


Fig. 3: The 3D and 2D interaction of target protein C-Raf and phytocompound withanone

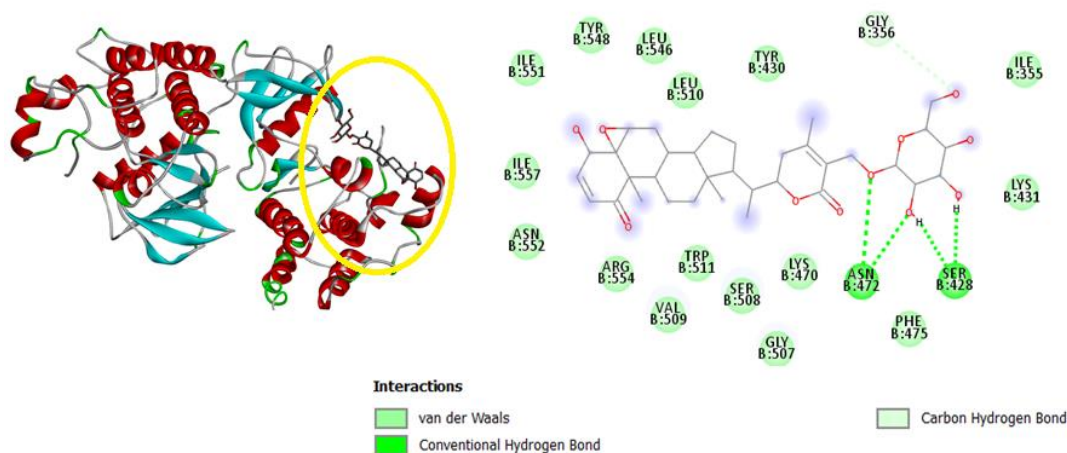


Fig. 4: 3D and 2D interactions of target protein C-Raf and phytocompound Sitoindoside IX

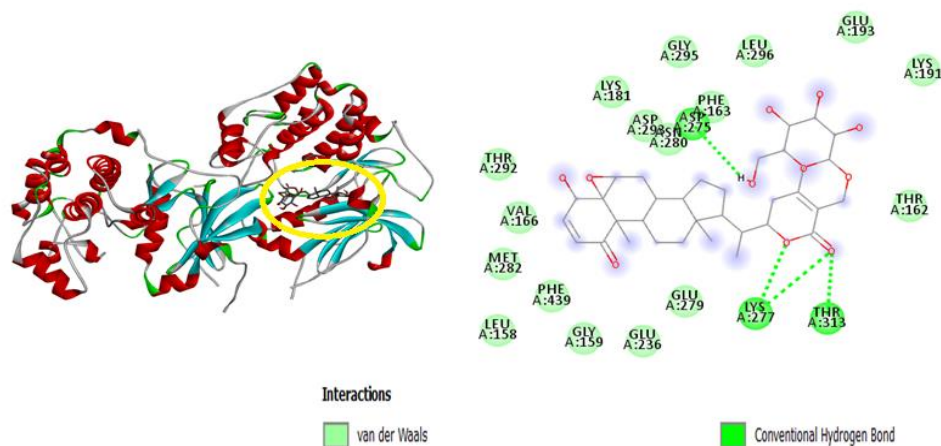


Fig. 5: 3D and 2D interaction of the target protein Akt 2 and the phytocompound Sitoindoside IX

Withanone and Sitoindoside IX showed good interaction with C-Raf with binding energy of -8.8 Kcal/mol and -8.7 Kcal/mol, respectively. Their interactions are depicted in Fig. 3 and Fig. 4. Sitoindoside IX and Withanone showed good interaction with Akt 2 with binding energy of -9.8 Kcal/mol and -9.5 Kcal/mol, respectively. Their interactions are depicted in Fig. 5 and Fig. 6. Sitoindoside IX, Withanone and Somniferine showed good interaction with GSK 3 β with binding energy of -

10.1 Kcal/mol, -9.7 Kcal/mol and -8.7 Kcal/mol respectively. Their interactions are shown in Fig. 7, Fig. 8 and Fig. 9. Anaferine and Isopelletierin did not show very significant interaction with the three proteins, C-Raf, Akt 1 and GSK 3 β . From these docking results, we found that Sitoindoside IX, Somniferine and Withanone were showing better docking scores with C-Raf, Akt 2 and GSK 3 β protein molecules, therefore, these phytocompounds can be used as potential anti-cancer drugs.

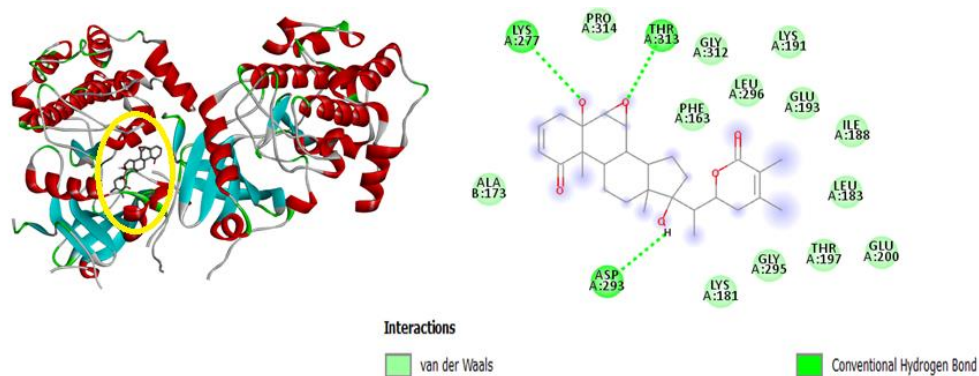


Fig. 6: 3D and 2D interaction of the target protein Akt 2 and the phytocompound Withanone

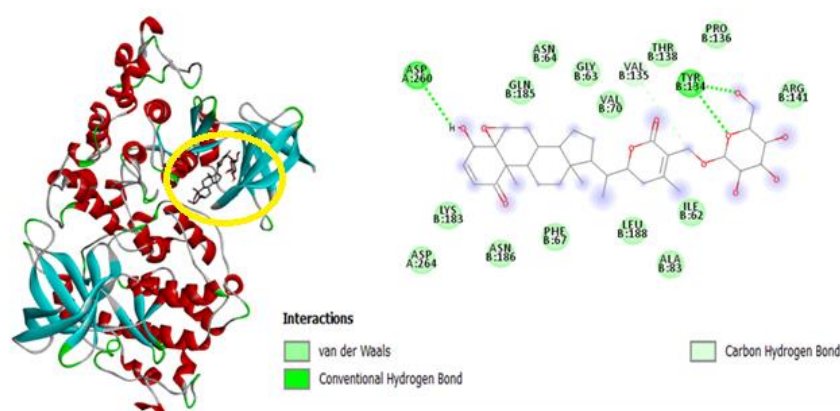


Fig. 7: 3D and 2D interaction of the target protein GSK 3β and the phytocompound Sitoindoside IX

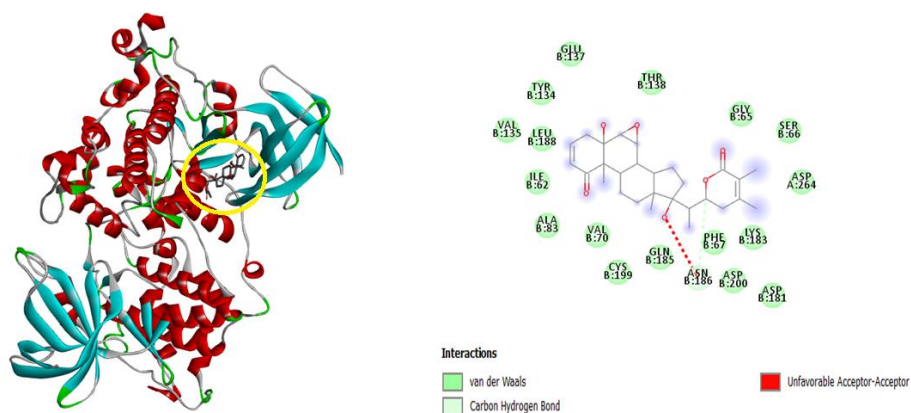


Fig. 8. 3D and 2D interaction of the target protein GSK 3β and the phytocompound Withanone

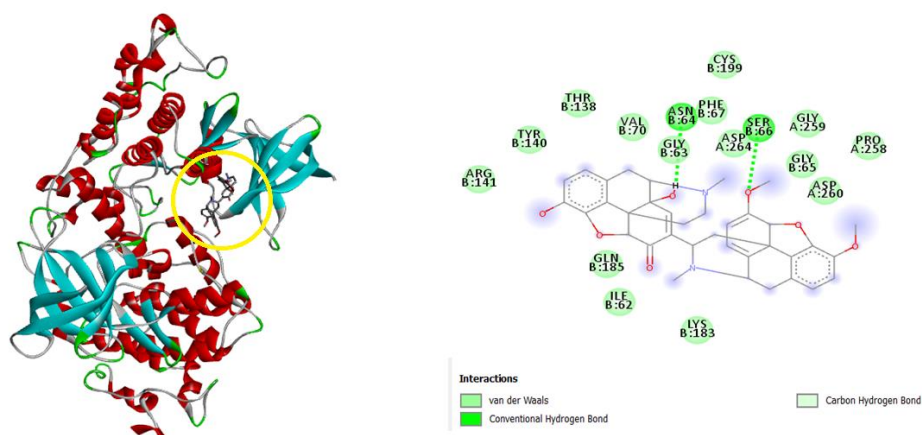


Fig. 9: 3D and 2D interaction of the target protein GSK 3β and the phytocompound Somniferine

DISCUSSION

Nature is blessed with many medicinal plants and the natural products are gaining importance in the discovery of anti cancer drugs. In this study we performed molecular docking for the phytocompounds of *Withania somnifera* with C-Raf, Akt 2 and GSK 3 β protein molecules. Reported studies revealed that the plant compounds like 2,ergost-25-ene-3,6-dione,5,12-dihydroxy-, (5.alpha., 12.beta), aspidospermidin-17-ol,1-acetyl-16-methoxy and 2-(3,4-dichlorophenyl)-4-[[2-[1-methyl-2-pyrrolidinyl] ethyl amino] -6-[trichloromethyl]-s-triazine had good binding affinity with breast cancer target protein (11). It has been reported that there are flavonoids available which are having anti-breast cancer activities, such as chrysin, flavonol, taxifolin (12). Understanding the potential of phytocompounds to develop anti-breast cancer drug, information about the compounds from *Withania somnifera* were retrieved from literature. The 2D and 3D structures of the phytocompounds were retrieved from PubChem and their binding patterns were explored against three selected target proteins involved in breast cancer.

In this present studies, Somniferine is better interacting with the protein C-Raf. The interaction between the amino acids in the binding site and the ligand includes conventional hydrogen bond, carbon hydrogen bond and vander waals force. Somniferine showed the best interaction with Akt 2 protein also. The interaction between the amino acids in the binding site and the ligand includes hydrogen bond, carbon hydrogen bond, vander waals force, alkyl bond and pi-alkyl bond. Finally, Sitoindoside IX showed the best interaction with GSK 3 β protein. The interaction between the aminoacids in the binding site and the ligand includes conventional hydrogen bond, carbon hydrogen bond and vander waals force.

CONCLUSION

Molecular docking results had shown their mode of binding with the target proteins through hydrogen bond and carbon hydrogen interactions. The binding energy values obtained from the docking and the interactions provide a primary evidence and scope for using Sitoindoside IX, Withanone, Somniferine phyto-compounds from *W. somnifera* against breast cancer. The above compounds showed better interaction with the chosen proteins and can be extensively studied to develop anti-breast cancer drugs. Also, these phytocompounds from *W. somnifera* can be considered effective against the target proteins. These computational results might be further confirmed by experimental animal models to develop targeted therapies against breast cancer.

CONFLICT OF INTEREST

The authors declare no conflict of interest

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