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Research Article

Withania Somnifera for Anticancer activity

Analysis Of Anticancer Activity And Its Molecular Interaction Mechanism Of Withanone, An Active Ingredient Of Withania Somnifera Using Molecular Docking

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Abstract: Withania somnifera is an annual evergreen shrub from the Solanaceae family, commonly known as Indian ginseng or Ashwagandha. The plant is mainly found in Asia and Africa regions. In the traditional Indian medicinal system ayurveda, Withania somnifera is used as a rejuvenator and sold in many countries as a dietary supplement. Withanolides are the major phytochemical constituent group found in the Withania somnifera, among which withaferin A and withanone, are considered to be major withanolides, which believed to be involved in majority of biological activity of Withania somnifera. Various studies including both in vitro and in vivo have reported regarding the anticancer potential of Withania somnifera. Along with the anticancer activity of W.somnifera, the anticancer efficacy of one of its major ingredients Withaferin A is also studied previously. This study aimed to analyse the anticancer potential of another major Withanolide present in W. Somnifera, Withanone. The study used Molecular Docking method to find the molecular binding affinity of Withanone towards various cancer proteins. The four major cancer proteins were B-cell lymphoma- extra large (Bcl-xL), Cellular FLICE (c-FLIP), Glutathione Reductase (GR) and Glutathione S- Transferases (GST). The protein structure obtained from the protein data bank and the structure of the molecule obtained from pubchem were modified and prepared for Docking studies with the help of MGL Tools. The protein ligand interaction study was conducted using the software, Autodock vina. The already known anticancer standard, 5-FluoroUracil is used as standard for comparison. Output obtained from the study is visualised using molecular visualiser tool, Pymol. Like the Withaferin A, Withanone also exhibited promising anticancer activity while studied using molecular docking methods.

Keywords: Withania somnifera, Withanone, Anticancer activity, Molecular Docking, Binding energy, cancer proteins

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1. INTRODUCTION

Ashwagandha, the commonly found Herbal ingredient in Indian system of medicine, is also known as Indian Ginseng, and was scientifically called the *Withania somnifera*, from the Solanaceae family. Since the extracts of *Withania somnifera* suggested to modulate various biological responses, it is extensively used in indigenous preparations for its properties like anti-ageing, aphrodisiac, cardiotonic, thyro-regulatory, antiperoxidative, anti-inflammatory, antitumor, anti-stress, anti-oxidant, immuno-modulatory, hemopoietic, and rejuvenating[1,2,3]. In kerala, Ashwagandha is used as a major herbal ingredient in many traditional medicinal formulations. In Ayurveda, *Withania somnifera* is provided as a Rasayana, tonic. There are Rasayana products like Ashwagandha Rasayana and aswagandhadi lehyam, in which the Ashwagandha is used as a major ingredient. Being a Rasayana formulation, the nutritional profiling and Heavy metal toxicity of Aswhwagandhadi lehyam is already studied[4]. While studying the Antidepressant potential of various medicinal plants, researchers have reported regarding the potential of *W.somnifera* also[5]. The anticancer studies about the *W.somnifera* has proven its capability to induce cytotoxicity in several human cancer cell lines. The mechanism behind the cytotoxicity is by the activation of both intrinsic and extrinsic apoptosis[6]. Withanolides are involved in various bodily functions such as anti-cancer activity[7]. Withaferin-A and withanone (withanolides), are the major biologically active constituents of Ashwagandha leaves[7,8] and believed to be involved in the majority of biological functions of Ashwagandha. In this study, it is aimed to analyse the anticancer potential of another major Withanolide present in *W. Somnifera*, Withanone. And also its molecular interaction mechaism. The pleiotropic mechanism of action of Withaferin A as a natural anticancer agent is already cited[9]. The targeting of TPX2-Aurora A complex by *Withania somnifera* derived Withanone is analysed by both computational and experimental methods evidenced to its anticancer activity[10]. Molecular Docking is an orientation prediction method, which predicts the preferred orientation, while two molecules (a protein and a ligand) bonds to form a stable compound[11]. In addition to prediction, it is very importantly used as a tool for interaction analysis studies, to understand the molecular interactions happening in biological science. In the field of drug designing, this advanced computer-aided technique is frequently used to evaluate the structure-based design in the bonding of small ligand molecules to the targeted protein[12]. There are studies reported regarding the anticancer activity of *Withania somnifera* and its active chemical constituents. Studies regarding the Molecular interactions of the major Withanolide, Withaferin A along with various cancer proteins were also going on the way. The compound of interest Withanone with molecular formulae $C_{28}H_{38}O_6$ with a molecular weight of 470.6g/mol were obtained from pubchem. The cancer proteins considered for the study Bcl-xL, c-FLIP, GR and GST were obtained from Protein Data Bank. The antiapoptotic protein, which down-regulates apotheosis, is a cancer cell considered in this study along with the known anticancer drug 5-Fluorouracil (5FU) for better comparison. The molecular docking study conducted for finding the binding affinity of Withanone with the four cancer proteins, have used software Autodock vina along with Autodock MGL tools. The outputs were visualised using the Pymol software to see a 3 diamentional view of molecular interaction. The well known anticancer drug 5-Fluorouracil, is also docked against all the four cancer proteins as a positive control to satisfy the study. The binding energy and the structural output of the study is analysed to find the anticancer potential of Withanone.

2. MATERIAL AND METHODS

Four major cancer proteins, B-cell lymphoma- Extra large (Bcl-xL), Cellular FLICE (c-FLIP), Glutathione Reductase (GR), Glutathione S- Transferases (GST) were obtained from Protein Data Bank (PDB) in pub format. Molecular structures of Withanone, from *Withania somnifera* were obtained from Pubchem in sdf format. The molecular structure of 5-Fluorouracil (5FU) is also obtained in sdf format from pubchem. In order to subject the proteins and ligands for molecular docking, the proteins preparation step was done by deleting water molecules in binding sites, adding H^+ ion and adding kolman charges, and later saved the same in pdbqt format using MGL tool software. As like the protein preparation step, the ligands (Withanone and 5FU) were also prepared initially using molecular visualiser tool Pymol to convert the sdf format to pdb format and later using MGL tools in AutoDock software 4.2 to prepare and keep in pdbqt format[13]. After preparation, the measurements of the size and position of binding site of cancer proteins were noted by finding the grid and the coordinates were noted as per the Lamarckian Genetic Algorithm and based on the same, the log file is created[14]. The docking study is done using the software Autodock vina and the results were obtained[15]. The result includes the log of binding affinity chart and also a pqbdt file which can be visualised as a 3D view of interaction. The binding energy is calculated with the equation:

$$\Delta G = (VL\text{-}L[bound] - VL\text{-}L[unbound]) + (VPP[\ bound] - VP\text{-}P[unbound]) + (VP\text{-}L[bound] - VP\text{-}L[unbound] + \Delta Sconf)$$

where,

P -The protein
L - The ligand
V - The pairwise evaluations
S - The loss of conformational entropy upon binding

3. RESULTS AND DISCUSSION

Anticancer activity of *Withania somnifera* by its binding affinity to the anti apoptotic proteins were analysed using the Docking. Studies aimed at the in vitro and in vivo validation of antiviral efficacy of the phytochemical, Withanone, are already studied[16,17]. Studies have already been established regarding the binding affinity of Withanone towards the ACE2-RBD complex[18].Molecular mechanism and therapeutic potential of a novel combination of cucurbitacin B and Withanone against induction of senescence in cancer were already reported. [19]

Table 1: Binding Energy ΔG (kcal/mol)		
Protein	**Withanone**	**5FU**
Bcl-xL	-12.9	-5.2
c-FLIP	-11.3	-5.1
GR	-14.1	-5.4
GST	-12.8	-5.3

The binding affinity is recorded by its binding energy (ΔG). The results were compared with the same of 5-FU, which is considered as standard. Table 1- details the most appropriate binding energy of Withanone with the four different protein molecules, in which the Withanone is found most active against the Glutathione Reductases, GR with a ΔG of -14.1 and secondly with Bcl-xL with ΔG -12.90. Previous studies which have reported the enhancement of glutathione by Withanone, supports our highest binding affinity value of Withanone against the Glutathione reductase[20]. The standard 5 FU also has a high affinity against these molecules with ΔG -5.40 and -5.20 respectively. Reports regarding the resistance to 5FU in colorectal cancer might be associated with the expression of endogenious Bcl-xL satisfies our obtained value of -5.2 as binding energy[21]. The output of AutoDock vina is tabulated with the highest affinity values and Binding energy (ΔG). The result thus compared with the result obtained from the affinity analysis of standard drug 5 FU also. All the output molecular structures were visualised using the Pymol software and the results were plotted in Figures 1 to 4.

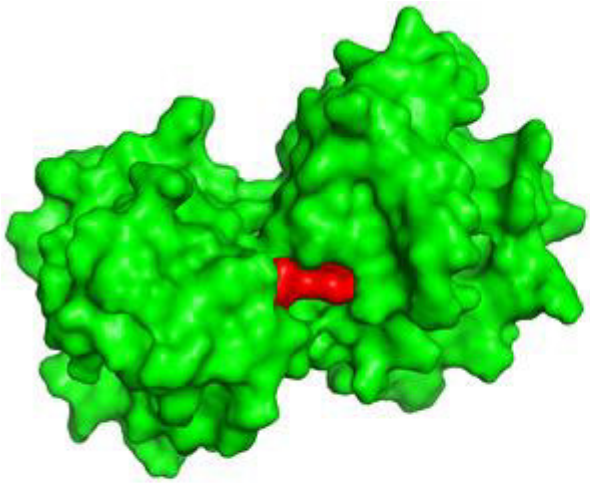

Fig 1: Bcl-xL - Withanone complex

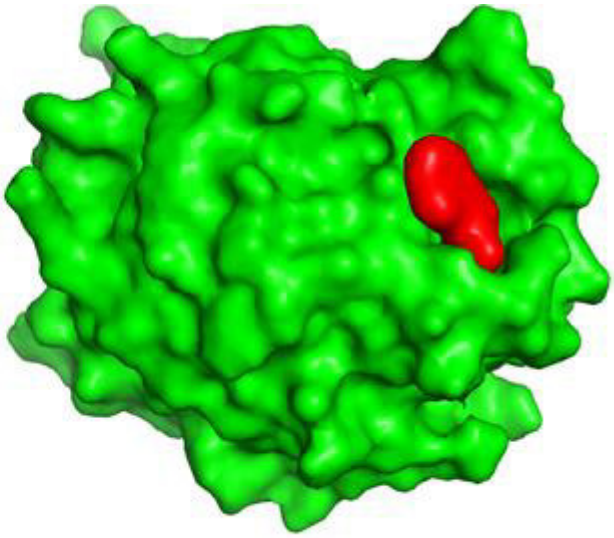

Fig 2: cflip - Withanone complex

Fig1 clearly visualises the bonding of Withanone with the Bcl-xL and fig 2 gives the structure of Withanone bonded with c-FLIP. Studies have already predicted regarding the interaction pattern with transmembrane protease serine and block the entry of SARS CoV-2 into cells[22]. Figure 1A and Figure 2A were providing the 3D structure of the interaction complex of 5FU molecules with the Bcl-xL and c-FLIP respectively.

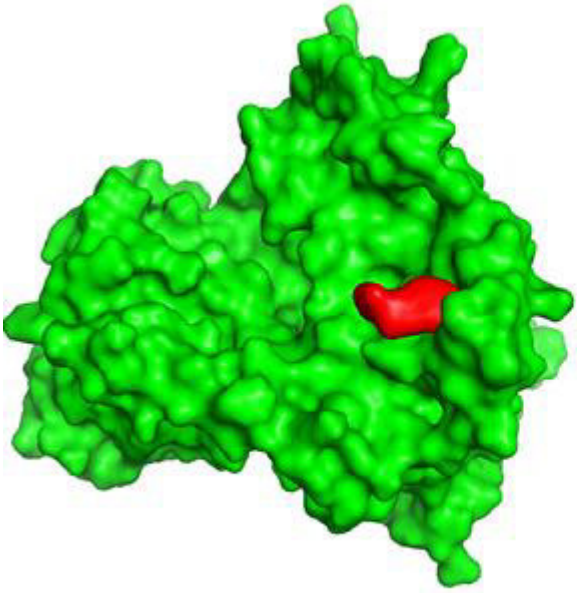

Fig 3: GR - Withanone complex

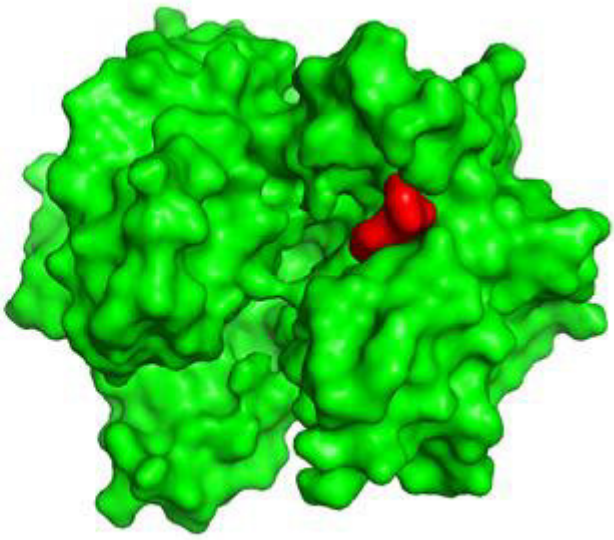

Fig 4: GST - Withanone complex

Figure 4 provides the molecular assembly of Withanone bonding with the Glutathione Reductase (GR) and the molecular bonding complex of Withanone with the Glutathione S-transferases (GST) is visualised in Figure 4. The bonding results of 5 FU with both GR and GST were detailed in the structural diagrams in Figure 3A and figure 4A.

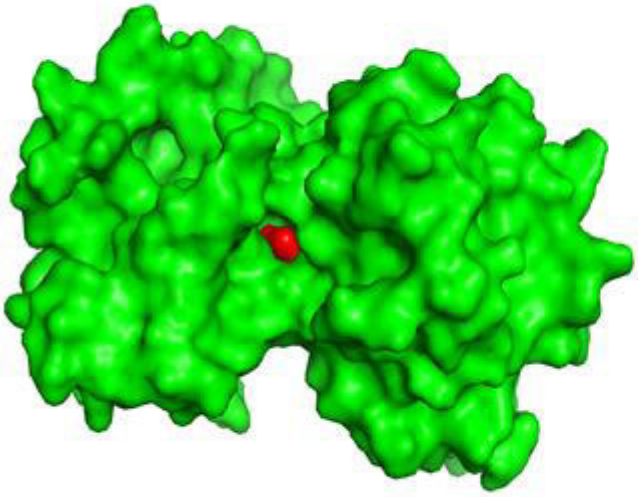

Fig 1A: Bcl-xL - 5FU complex

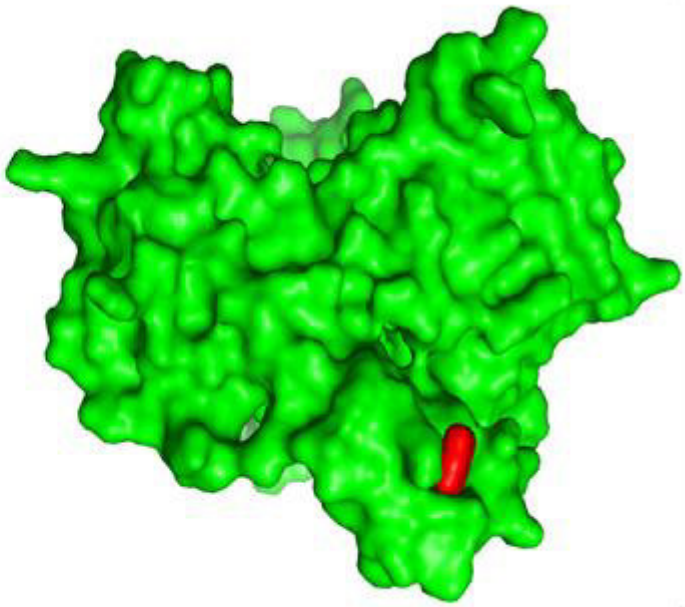

Fig 3A: GR - 5FU complex

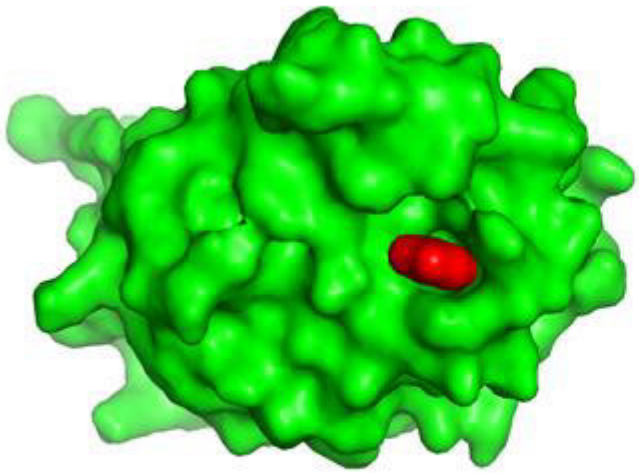

Fig 2A: cflip - 5FU complex

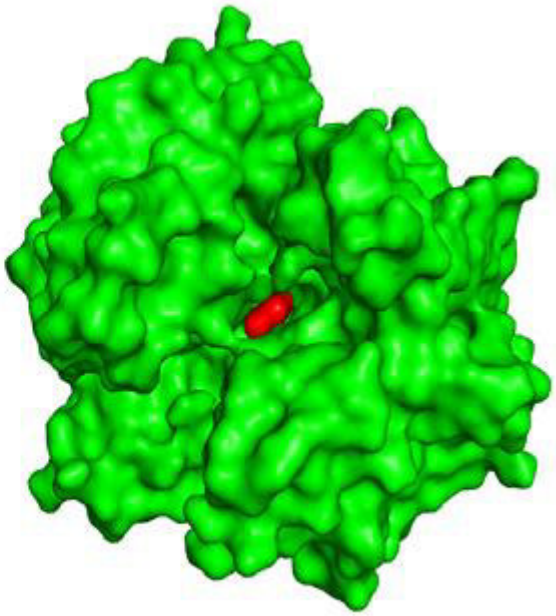

Fig 4A: GST - 5FU complex

The efficacy of Withanone, a major phytochemical in the Withanolide group of *Withania somnifera* is studied using the molecular docking mechanism and the results were obtained. The role of withanone in abrogation of mortalip-p53 complex and re-activation of p53 is a significant function in the cancer therapy[23]. Our study regarding the molecular interaction of withanone with various cancer proteins, also satisfies to the importance of withanone in cancer therapy. Liu K, have reported that the anticancer efficacy of a compound is measured generally by its capability to induce apoptosis, which is the genetically regulated cell death, in a cancer cell[24]. The ability of docking in finding the interaction property and also predicting the binding site of the target protein, which will intern help in drug designing is detailed[25]. In the anticancer activity analysis of withanone, the capability of withanone in inhibiting the anti apoptotic Survivin, and activated anticancer mechanism is already studied and reported can be considered in support of our study[26]. The binding affinity of Withanone against all proteins ranged between -14.1kcal/mol and -11.3 kcal/mol. but the binding affinity of 5FU were four between -5.4kcal/mol and -5.1kcal/mol only. The anti apoptotic activity of Bcl-2 family protein, Bcl-xL (B-cell lymphoma-extra large), and its capsize activation and regulation of programmed cell death is also recorded[27]. The Withanone has a binding affinity of -12.9kcal/mol with the Bcl-xL protein. Among 10 various binding combinations, most have provided similar binding affinity with no significant variations. The anticancer drug, 5-Fluorouracil has also been studied in this regard and provided an affinity of -5.20kcal/mol. Studies on c-FLIP as the most noted anti apoptotic protein as it suppresses both the chemotherapy and cytokine induced apoptosis[28]. Withanone have exhibited a comparatively lesser binding affinity of -11.3kcal/mol against c-FLIP, where 5FU have only recorded -5.1kcal/mol. The catalysis activity of Glutathione reductase, in catalysing the reduction of glutathione disulphide to a trimeric protein complex GSH, which plays an important role in cell proliferation and apoptosis was studied[29]. Among the four cancer proteins, Withanone, exhibited the highest affinity against the GR, which is -14.1kcal/mol. The role of Glutathione S-transferases (GST) in GSH and xenobiotic substrate conjugation catalysis is studied[30]. The binding affinity of Withanone towards the GST is -12.8kcal/mol while 5FU have reported a binding affinity of -5.3 kcal/mol

4. SUMMARY AND CONCLUSION

The Anticancer activity of *Withania somnifera* is already studied. Being an important phytochemical constituent of the commonly used herb *Withania somnifera*, Ashwagandha, the Withanone which is included in the Withanaloid group is also studied for its efficacy. To draw a primary attention towards the efficacy analysis of Withanone, the study is done with the help of molecular Docking, where the binding affinity of molecules towards various cancer proteins found promising results. The binding energy of Withanone with all studied proteins was below -11kcal/mol and against Glutathione Reductase it was found to be -14.1kcal/mol. Since the compound has exhibited a very promising result in Docking studies, its further in vitro and invivo studies need to be focussed, to see how it can be used as a chemotherapeutic drug, as the requirement for herb based drugs are increasing nowadays.

5. ACKNOWLEDGEMENT

Dr. Arun and Dr. Sadagopan, of Bharathiar University has helped in explaining technicalities of molecular docking procedures.

6. AUTHOR CONTRIBUTION STATEMENT

Madhan Shankar has guided, supervised and encouraged Ben Raj in the findings of this work. Both the authors discussed the results and contributed equally to the final manuscript.

7. CONFLICT OF INTEREST

Conflict of interest declared none.

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