

# Activities of Root-Derived Phytochemicals of *Withania Somnifera* on Cancer Kinase: Computational Approach

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**ABSTRACT:** Cancer is an uncontrolled growth of abnormal cells in the body. Cancerous cells are malignant in nature and kinase enzyme is essential for the survival and/or proliferation of the cancer cell. In this context, kinase inhibitors have been extensively investigated as anti-cancer drugs. In the presence of kinase inhibitors, the cancer is unable to proliferate or survive. Natural compounds are important sources of drugs. The present investigation concentrates on discovering anti-cancer compounds that are present in *Withania somnifera*, a medicinal plant belonging to Solanaceae family. In the current study, phytochemicals of *Withania somnifera* (Root) is identified using Gas Chromatography-Mass Spectroscopy analysis (GC-MS) to dock against the enzyme cancer kinase (PDB ID: 3COI-Crystal structure of p38delta kinase) using iGEMDOCK v2.5.0. The results of enzyme-substrate interaction energies show that these compounds are active against cancer kinase. Oleic acid, a phytochemical of *Withania somnifera* gave best binding energy of -84.26 kcal/mol and showed low toxic hazard with class I in Toxtree v2.5.0. Further research on *Withania somnifera* will be useful in drug designing against cancer.

**KEYWORDS:** Cancer kinase, Phytochemicals, *Withania somnifera*, Drug designing.

## I. INTRODUCTION

Drug discovery in the area of Oncology has benefited significantly right from progress in understanding how to target kinases with small molecules relative to other disease indications. One reason for this is that many kinases have been found to be intimately involved in the processes leading to tumour cell proliferation and survival [1]. First, there are kinase targets that have become impervious to normal regulatory mechanisms following genetic mutation or translocation. These kinases have transforming capacity and are therefore considered to be oncogenic. The constitutive activity of this class of kinase target makes them essential for survival and/or proliferation of the cancer cell [2]. This so-called oncogene addiction renders the cancer cell exceptionally susceptible to the appropriate kinase inhibitor. The success of mutationally marked kinases as drug targets has motivated an intensive effort to survey the kinome across a broad range of tumour types for mutations [3]. These studies have uncovered a large number of kinases bearing mutations, which are currently in the process of being functionally characterized. This study aims to screen for inhibitors of cancer kinase so that the proliferation of cancer cells can be stopped.

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a)

Fig.1. a) *Withania somnifera*

## II. LITERATURE SURVEY

*Withania somnifera* (Figure 1) is commonly known as Ashwagandha, It is an Indian Ginseng and is a member of the Solanaceae family [4]. *Withania* plant is used for its anticancer properties, anti-inflammatory, and fertility, food for cattle and in making chutney [5]. The roots of *Withania somnifera* consist primarily of compounds known as withanolides, which are believed to possess extraordinary medicinal properties. Withanolides are steroidal and bear a resemblance, both in their action and appearance, to the active constituents of Asian ginseng (*Panax ginseng*) known as ginsenosides. Ashwagandha's withanolides have been researched in a variety of animal studies examining their effect on numerous conditions, including immune function and even cancer [6].

Ashwagandha is reported to have anti-carcinogenic effects. Research on animal cell cultures has shown that the herb decreases the levels of the nuclear factor kappa B, suppresses the intercellular tumor necrosis factor, and potentiates apoptotic signalling in cancerous cell lines [8]. One of the most exciting of the possible uses of Ashwagandha is its capacity to fight cancers by reducing tumor size [9, 10]. To investigate its use in treating various forms of cancer, the antitumor effects of *Withania somnifera* have been studied by many researchers. In one study, the herb was evaluated for its anti-tumor effect in urethane-induced lung tumors in adult male mice [11, 12]. Following administration of Ashwagandha over a period of seven months, the histological appearance of the lungs of animals which received the herb was similar to those observed in the lungs of control animals.

The objectives of this work is to study the role of kinase in cancer, docking studies to be performed with plant based compounds and to evaluate the binding efficiency of the selected lead molecule with reference Ligand.

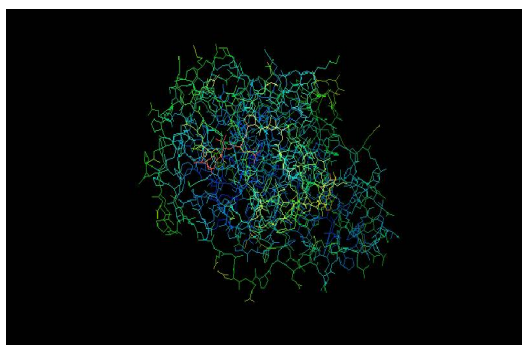
Target selected for investigation is cancer kinase (PDB ID: 3COI- *Crystal structure of p38delta kinase*) (Figure 2) [13] and the ligands are those compounds which are obtained from *Withania somnifera*- Root (Table 1). The compounds from *Withania somnifera* - root were extracted and their respective ligand downloaded from PUBCHEM database. Cancer kinase (3COI) was got downloaded from Protein data bank database and energy minimized using Universal force field. Using iGEMDOCK 2.1 docking software [14] the compounds of *Withania somnifera* were docked against cancer kinase.

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Fig.2.a) 3COI- Crystal structure of P38 delta Kinase

**Table 1: List of the phytochemicals in *Withania somnifera* - root obtained from PUBCHEM database**

NAME OF THE COMPOUND	PUBCHEM ID
Propane, 1,1-diethoxy-2-methyl-	519415
Butane, 1,1-diethoxy-2-methyl-	19695
2-Nonanone	13187
Amyl Nitrite	10026
Dodecanoic acid	3893
3-tert-Butyl-4-hydroxyanisole	8456
Tetradecanoic acid	11005
n-Hexadecanoic acid	985
Decanoic acid, ethyl ester	8048
9-Octadecenal	5283381
1-Tridecyne	117754
Oleic acid	445639

The 12 photochemical represented in the table were confirmed to be present in *Withania somnifera* when analysed in the PUBCHEM database.

### III. MATERIAL AND METHODS

#### Preparations

The three dimensional structure of target cancer Kinase (PDB ID: 3COI- *Crystal structure of p38delta kinase*) was retrieved from protein data bank at 1.3 Å RMSD resolution and active sites were analysed using Q-SiteFinder [15]. The compounds obtained from *Withania somnifera* were identified by GC-MS method [5]. The 2D structures of Phytochemicals from *Withania somnifera* activity were retrieved from PUBCHEM compound database to determine anti-cancer and then with the help of Open Babel software [16] these .sdf format files were converted to .mol format files and energy minimized using Universal Force Field (UFF).

#### Docking

The ligands and crystallographic water molecules were removed from the protein and the chemistry of the protein was corrected for missing hydrogen. Following the above steps of preparation, the protein was subjected to energy minimization using the Universal Force Field. Q-Sitefinder server was used to detect the active sites and docking was

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performed by iGEMDOCK molecular docking software. Docking was performed with all the potential active sites detected on Cancer kinase enzyme. During docking, at first the molecules were prepared and bonds, bond orders, explicit hydrogen's, charges, flexible torsions were assigned to both the protein and ligands. From the docking wizard, ligands were selected and the scoring function used is iGEMDOCK score. If hydrogen bonding is possible, the hydrogen bond energy contribution to the docking score is assigned a penalty based on the deviations from the ideal bonding angle. Using this option, it is possible to reduce the number of unlikely hydrogen bonds reported also internal electrostatic interaction; internal hydrogen bond sp<sup>2</sup>-sp<sup>2</sup> torsions are calculated from the pose by enabling the ligand evaluation terms.

The search algorithm is taken as iGEMDOCK and number of runs taken are 10 and max interactions were 2000 with population size 200 and with an energy threshold of 100 also at each step least 'min' torsions/translations/rotations are tested and the one giving lowest energy is chosen. If the energy is positive (i.e. because of a clash or an unfavourable electrostatic interaction) then additional 'max' positions will be tested. If the pose being docked is closer to one of the ligands in the list than specified by the RMSD threshold, an extra penalty term (the Energy penalty) is added to the scoring function. This ensures a greater diversity of the returned solutions since the docking engine will focus its search on poses different from earlier poses found. The energy penalty was set to 100, RMSD threshold was 2.00 and RMSD calculation by atom ID (fast) were set. Docking was conducted between Protein and Inhibitor which results in binding affinities in kcal/mol and docking run time. The Phytochemical which gives lowest binding energy is chosen as the best inhibitor. iGEMDOCK showed better overall performance in docking simulations when compared with other software.

### Adverse effect prediction

Toxicity property was estimated using Toxtree v2.5.0 to know whether those phytochemicals have the potential of adverse toxicity effect in humans [17] (Table 4).

**Table 2: Physicochemical properties of compounds from *Withania somnifera***

Compound name	Molecular formula	Molecular weight [g/mol]	XLogP3	H-bond donor	H-bond acceptor
Propane, 1,1-diethoxy-2-methyl-	C <sub>8</sub> H <sub>18</sub> O <sub>2</sub>	146.22732	2.2	0	2
Butane, 1,1-diethoxy-2-methyl-	C <sub>9</sub> H <sub>20</sub> O <sub>2</sub>	160.2539	2.5	0	2
2-Nonanone	C <sub>9</sub> H <sub>18</sub> O	142.23862	3.1	0	1
Amyl Nitrite	C <sub>5</sub> H <sub>11</sub> NO <sub>2</sub>	117.14634	1.8	0	3
Dodecanoic acid	C <sub>12</sub> H <sub>24</sub> O <sub>2</sub>	200.31776	4.2	1	2
3-tert-Butyl-4-hydroxyanisole	C <sub>11</sub> H <sub>16</sub> O <sub>2</sub>	180.24354	3.2	1	2
Tetradecanoic acid	C <sub>14</sub> H <sub>28</sub> O <sub>2</sub>	228.37092	5.3	1	2
n-Hexadecanoic acid	C <sub>16</sub> H <sub>32</sub> O <sub>2</sub>	256.42408	6.4	1	2
Decanoic acid, ethyl ester	C <sub>12</sub> H <sub>24</sub> O <sub>2</sub>	200.31776	4.6	0	2
9-Octadecenal	C <sub>18</sub> H <sub>34</sub> O	266.46196	7.2	0	1
1-Tridecyne	C <sub>13</sub> H <sub>24</sub>	180.32966	6.2	0	0
Oleic acid	C <sub>18</sub> H <sub>34</sub> O <sub>2</sub>	282.46136	6.5	1	2

The physicochemical properties in *Withania somnifera* was studied. The molecular number, molecular weight, XlogP3 values were identified. It is also analysed that most of the compounds have 2 hydrogen bond acceptors.

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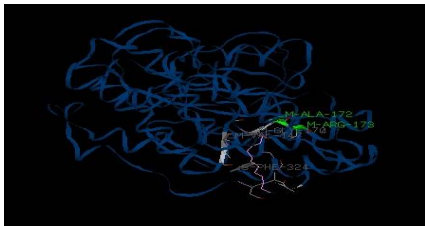
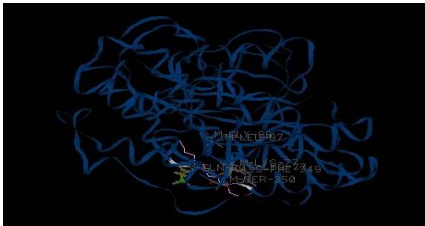
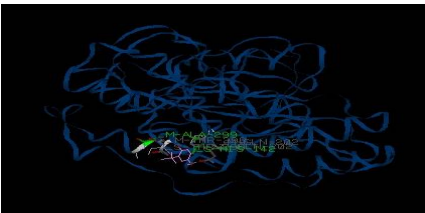
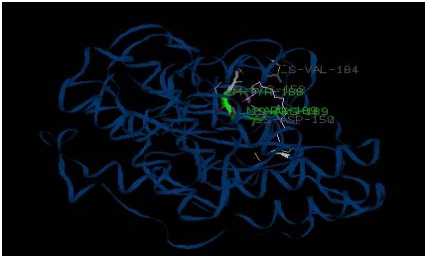
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## IV. EXPERIMENTAL RESULTS

Table 3: Docking results with the compounds and its pose energy

Docking results	Compound/ Phytochemical	Pose energy (kcal/mol)
	Propane, 1,1-diethoxy-2-methyl-	-55.18
	Butane, 1,1-diethoxy-2-methyl-	-57.07
	2-Nonanone	-59.17
	Amyl Nitrite	-61.18
	Dodecanoic acid	-72.91

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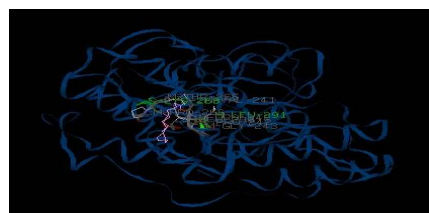
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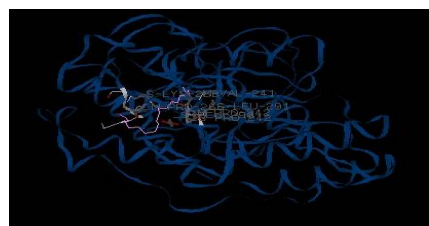
3-tert-Butyl-4-hydroxyanisole

-67.35



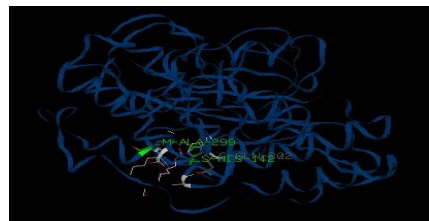
Tetradecanoic acid

-79.64



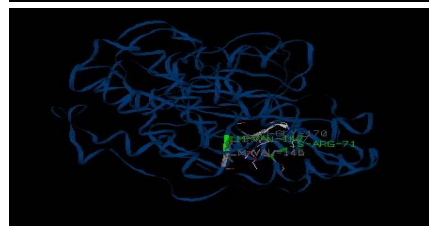
n-Hexadecanoic acid

-84.12



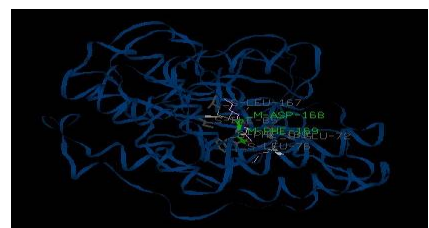
Decanoic acid, ethyl ester

-66.72



9-Octadecenal

-79.40



1-Tridecyne

-71.10

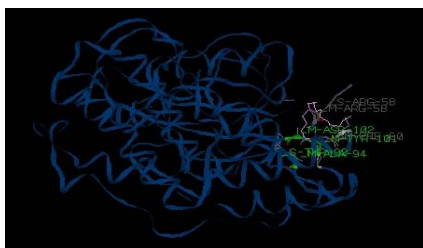


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Oleic acid

-84.26

Docking of the phytochemicals was done. It is analysed that Oleic acid and n-Hexadecanoic acid have high pose energy of 84.12 and Propane, 1,1-diethoxy-2-methyl- have low pose energy of -55.18.

**Table 4: Toxic hazard estimation using TOXTREE v2.5.0**

Compound name	Toxic hazard
Propane, 1,1-diethoxy-2-methyl-	Low (Class I)
Butane, 1,1-diethoxy-2-methyl-	Low (Class I)
2-Nonanone	Intermediate (Class II)
Amyl Nitrite	High (Class III)
Dodecanoic acid	Low (Class I)
3-tert-Butyl-4-hydroxyanisole	Low (Class I)
Tetradecanoic acid	Low (Class I)
n-Hexadecanoic acid	Low (Class I)
Decanoic acid, ethyl ester	Low (Class I)
9-Octadecenal	Low (Class I)
1-Tridecyne	High (Class III)
Oleic acid	Low (Class I)

The toxic hazard estimation of the phytochemicals in *Withania somnifera* was done by TOXTREE v.2.5.0 and it was figured out that Amyl nitrite and 1-Tridecyne have High (class III) toxicity.

Molecular docking predicts the binding affinity of the Ligand to the protein based on complex geometry. If an effective inhibitor, or drug, can be found to stop the kinase from carrying out its functions of a cancer cell, its survival and/or proliferation could be stopped. In the current study, the compounds of *Withania somnifera* were docked against cancer kinase using iGEMDOCK 2.1. Physiochemical properties (Table 2) of compounds from *Withania somnifera* were checked and results of docking were shown (Table 3). In the present study, Oleic acid, the compound of *Withania Somnifera* got best ligand pose energy of -84.26 kcal/mol. Further research can be conducted on *Withania Somnifera* to explore more data to inhibit cancer kinase.

### V. CONCLUSION

1. The best binding phytochemicals of *Withania somnifera* were identified by binding energies obtained in docking studies of phytochemicals with cancer kinase (3COI).
2. The docking studies are also helpful for understanding the binding mode and interaction of Phytochemicals of *Withania somnifera* with cancer kinase (3COI).
3. The present study also provides an insight into the mechanism of action of Phytochemicals against diseases.

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4. This in-silico approach can be further investigated to generate more effective and potential cancer kinase inhibitors through ligand based drug designing approaches.
5. Hence, it can be concluded that phytochemicals of *Withania somnifera* can act as cancer kinase inhibitors. Further, it was found that Oleic acid of *Withania somnifera* can effectively inhibit cancer kinase (3COI).

### REFERENCES

- [1] Futreal P A, Lachlan C, Marshall M, Down T, Hubbard T, Wooster R, Rahman N, Michael R. Stratton, "A census of human cancer genes". Nature Rev Cancer 2004; 4:177-183.
- [2] Weinstein I.B., Begemann M, Zhou P, Han E.K , Sgambato A, Doki Y, Arber N, Ciaparrone M and Yamamoto H , "Disorders in cell circuitry associated with multistage carcinogenesis: exploitable targets for cancer prevention and therapy", Clin Cancer Res 1997; 3: 2696-2702.
- [3] Weinstein I.B and Joe A.K, "Mechanisms of disease: Oncogene addiction - a rationale for molecular targeting in cancer therapy", Nature Clin Pract Oncol 2006; 3: 448-457.
- [4] Forbes S, Clements J, Dawson E, Bamford S, Webb T, Dogan A, Flanagan A, Teague J, Wooster R, Futreal P.A and Stratton Cosmic M.R, Br J Cancer 2005; 94: 318-322.
- [5] Senthil Kumar M., Vinoth Kumar D, Saravana Kumar A, Aslam A and Shajahan A, "The Phytochemical Constituents of *Withania somnifera* and *Withania obtusifolia* by GCMS Analysis", International Journal of Pharmacognosy and Phytochemical Research 2011; 3(3): 31-34.
- [6] Panneerselvan K, Jayapragasam M, Mani D.K and Bhavanisankar K, "Indigenous knowledge of *Withania obtusifolia* in Trichy region", World Ayurveda summit in Bangalore, 2003.
- [7] <http://ashwagandha.co.in/>
- [8] Grandhi A, A comparative pharmacological investigation of Ashwagandha and Ginseng, Ashwagandha Journal of Ethnopharmacology 1994; 3: 131-135.
- [9] Ichikawa H, Takada Y, Shishodia S, Jayaprakasam B, Nair M.G and Aggarwal B.B, Molecular Cancer Therapeutics 2006; 5:1434-1445.
- [10] Prakash J, S.K. Gupta and A.K. Dinda, *Withania somnifera* root extract prevents DMBA-induced squamous cell carcinoma of skin in swiss albino mice, Nutr Cancer 2002; 42(1): 91-97.
- [11] Jayaprakasam B, Zhang Y, Seeram N and Nair M., Antiproliferative activity of *Withania somnifera* leaf components on human tumor cell lines and their structure activity relationships, Life Science 2003; 74: 125-132.
- [12] Singh N. Singh S.P, Nath R, Singh D.R, Gupta M.L, Kohli R.P and Bhargava K.P , Prevention of Urethane induced lung adenomas by *Withania somnifera* (L.) dunal in albino mice ., International Journal Crude Drug Research 1986; 24: 90-100.
- [13] RasWin Molecular Graphics (<http://www.rasmol.org/>) Windows version 2.7.5.2.
- [14] iGEMDOCK V2.1 (<http://gemdock.life.nctu.edu.tw/dock>)
- [15] Q-SiteFinder Ligand Binding Site Prediction [<http://www.modelling.leeds.ac.uk/qsitefinder/>]
- [16] Open Babel [[http://openbabel.org/wiki/Main\\_Page](http://openbabel.org/wiki/Main_Page)]
- [17] Toxtree (Estimation of Toxic Hazard - A Decision Tree Approach) (<http://toxtree.sourceforge.net>)