

**STUDIES ON WITHANOLIDES FROM *WITHANIA SOMNIFERA*:  
*IN SILICO* ELUCIDATION OF THEIR ACTION MECHANISMS  
AND ENGINEERING OF THEIR BIOSYNTHETIC PATHWAY**

by

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## CERTIFICATE

This is to certify that the thesis entitled “**Studies on withanolides from *Withania somnifera*: In silico elucidation of their action mechanisms and engineering of their biosynthetic pathway**” being submitted by **Mr. Abhinav Grover** to the Indian Institute of Technology, Delhi, for the award of the degree of “Doctor of Philosophy”, is a record of the bonafide research work carried out by him, which has been prepared under our supervision in conformity with the rules and regulations of the Indian Institute of Technology, Delhi. The research reports and the results presented in this thesis have not been submitted for any degree or diploma in any other University or Institute.

**Dr. D. Sundar**

**Prof. V. S. Bisaria**

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## ABSTRACT

*Withania somnifera* commonly known as Ashwagandha, which belongs to the Solanaceae family, is held in high repute in traditional Indian medicine, largely due to the presence of steroidal lactone phytochemicals collectively known as withanolides such as withaferin A, withanone, withanolide A, withanolide D and many more. Recent surge towards usage of high specificity drugs having reduced side effect profile is urging explorations with these naturally occurring herbal drug candidates. In the recent past, various crucial targets of withanolides have been reported like NF- $\kappa$ B, proteasomes, heat shock protein, mortalin and acetylcholinesterase among others. The inhibition of these receptors can lead to prevention/cure of dreadful diseases like cancer and Alzheimer's. Though the therapeutic effects of these withanolides have been well documented, the detailed specific binding modes on the respective disease-associated targets are still unknown. In the present study, computational tools including homology modeling, molecular docking and molecular dynamics simulations were used to elucidate the interactions between the drug compounds and their target molecules and to identify the stability of such interactions. Molecular docking studies were carried out to locate the binding modes and to determine the binding affinities. The interactions between substrates (withanolides) as ligands and their macromolecular receptors of known/modeled three dimensional structures were simulated by molecular dynamics approach thus accounting the flexibility of the macromolecules as well as of the ligands. The molecular interactions patterns like H-bonding and van der Waals interactions were studied to elucidate the binding modes. Cluster analysis was also performed to determine the frequency of docked structures with the particular conformations.

Another important issue concerning these withanolides is related to their production. It is not an easy task to produce these compounds economically by extraction from intact plants and meet the ever-increasing demands. The concentration of withanolides in plants is generally low, the gestation period of the plant is very long (5-7 years) and the accumulation pattern of these compounds is highly susceptible to geographical and environmental conditions. The concentration of withanolides usually ranges from only 0.001 to 0.5% of dry weight in leaves and roots of the plant. The chemical synthesis is also not economically feasible, as it yields only restricted quantities at high costs. Plant tissue culture technology appears as a viable and attractive route for increased production of these pharmacologically active metabolites which provides high productivities, efficient downstream recovery and additional advantage of invoking controls and regulations during the production stage. The ability to introduce foreign DNA into plant cells has come up as one of the significant advances in plant biotechnology, as it allows enhancement in the required production traits in the plants. The enzyme squalene synthase, which is commonly depicted as the incipient and crucial branch point enzyme of the isoprenoid pathway to sterol biosynthesis, has attracted considerable interest as a potential regulatory point that controls carbon flux into sterols. In the present study, in order to enhance the inherent withanolide content, cDNA of this key regulatory enzyme was isolated from the leaves of *Withania somnifera*. Then using *Agrobacterium tumefaciens* as a transformation vehicle, we developed recombinant cell lines of *Withania somnifera* transformed with squalene synthase were developed. A significant enhancement in the squalene synthase activity and withanolide content was observed in the recombinant cell line as compared to the non-transformed cell line.

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