Insilico Studies of Thymoquinone in *Nigella sativa* as Potential Antitumor Agent

Nivethitha S[1], Rashmi S[1], Hemalatha CN[1] and Vijey Aanandhi M[1]

[1]Department of Pharmaceutical Chemistry and Analysis, School of Pharmaceutical Sciences, Vels University, (VISTAS), Chennai

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Corresponding Author

Vijey Aanandhi M. E-mail:*hodpchemistry@velsuniv.ac.in*

ABSTRACT

Introduction and Aim: The objective of this study was to study the binding energy of *N. sativa* biological active compounds, and drug likeliness by *insilico* techniques for anticancer activity. Thymoquinone inhibited vascular endothelial growth factor–induced extracellular signal-regulated kinase (ERK) activation. The proteins were retrieved from PDB bank and plant data compounds are taken from literature survey and chosen 4 compounds such as thymoquinone, alpha-hederin, dithymoquinone and thymohydroquinone for the study.

Materials and Methods: Auto dock 1.2.6 software is a suite of automated docking tools. It is designed to predict how small molecules, such a substrate or drug candidates, bind to receptors of the known 3d structure. 2XIR, 1NME, 1Q0R and IR9O protein preparation and optimization, ligand preparation and optimization and docking simulations were carried out by using biological databases like PubChem, Drug Bank, Protein Data Bank. These compounds are visualized by using Discovery studio 4.1 Visualizer.

Results: From the docking results , thymoquinone was showing satisfactory dock score values and it also satisfied the Lipinski's rule of five for drug likeness.

Conclusion: Present study indicates that thymoquinone inhibits tumor angiogenesis and tumor growth and could be used as a potential drug candidate for cancer therapy.

Key Words: *Nigella sativa*, thymoquinone, ERK, AKT pathway, AutoDock 4.2, Discovery Studio Visualizer 4.1.

INTRODUCTION

Spices and medicinal herbs are known to have anti-cancer characteristics which can be used to target the tumor growth and further damage (1). One such spice is *Nigella sativa*, also called as black cumin. *Nigella sativa* (*N. sativa*) is the annual plant of the family Ranunculaceae is a widely used medicinal plant throughout the world. It is very popular in various traditional systems of medicine like Unani, Ayurveda and Siddha. It has been widely used as antihypertensive, liver tonics, diuretics, antidiarrheal, apetite stimulant, analgesics, antibacterial and skin disorders (2). It is listed in the "Medicine of the Prophet" as a natural remedy to cure all pathological conditions. The main active component of black cumin volatile oil is Thymoquinone (TQ), of about 54% and others include monoterpenes like p-cymene, dithymoquinone (TQ2), thymohydroquinone (THQ) and alpha hederin (3). Extensive studies on *N. sativa* have been carried out by various researchers and a wide spectrum of its pharmacological actions have been explored which may include antidiabetic, anticancer, immunomodulator, antimicrobial, anti-inflammatory, spasmolytic, bronchodilator, hepato-protective, renal protective, gastro-protective, antioxidant properties (4). The seeds

Figure 1: *Nigella sativa* flower

Figure 2: Seeds

are thought to contain active ingredients having antitumor characteristics.

Thymoquinone is a principle component in Black cumin inhibits the DNA synthesis process leading to apoptosis and thereby hindering the cell proliferation. 5-fluorouracil inhibits thymidine synthesis and is seen to cure many forms of cancer. A number of studies have established that the pro-apoptic mechanism of thymoquinone is mediated by both p53 dependent pathways and p53 independent pathways (5). The Anti-tumor effects of Black cumin is because of its potent anti-inflammatory properties. This was established by Chehl N *et al* (6), a research study on thymoquinone on pancreatic cells, in which it was observed that this compound inhibits NF-κβ pathway leading to reduction in the growth of pancreatic cancer. And also, the anticancer effect of Black cumin is because of its anti-angiogenic mechanism. In this content, it was observed that thymoquinone inhibits its AKT and EKT signalling pathways and depicts inhibitory action on key cancer regulatory proteins (7).

MATERIALS AND METHODS

The protein and ligand interactions takes an important part in protein function. Both ligand and its binding site are essential components for understanding how the protein-ligand complex functions. Molecular docking is a key tool in structural molecular biology and computer assisted drug design (8).

Black cumin has number of chemically diverse agents contributing to its anti-cancer functionality. Literature review revealed the principle bioactive components of black cumin i.e. Thymoquinone, alpha hederin, dithymoquinone and thymohydroquinone and their structures are given in Table No.1. Lipinski's properties such as molecular weight, log p, molar refractivity, number of hydrogen bond acceptors and donors taken from SCFBio software for thymoquinone and it is satisfied the Lipinski's rule of five for drug likeness. The values of the Lipinski's properties are highlighted in Table: 2.

Protein Preparation

The three-dimensional coordinates of the crystal structure of the proteins (PDB-ID: 2XIR, 1NME, 1Q0R and IR9O). were downloaded from the RCSB protein data bank archive and used for docking studies. The protein structures were prepared in order to obtain the correct ionization and tautomeric state of amino acids residues. Further, water molecules were removed and polar hydrogen atoms were added. Then, the kollman united atom partial charges and salvation parameters were assigned. The protein preparation process resulted in a PDBQT file that contained Autogrid and Autodock.

Ligand Prearation

The three-dimensional structures of small molecules were prepared by identifying the root and its expansion, as required by the docking programs.

Ligand Docking

AutoDock is a tool used for predicting the interactions between the receptor (macromolecule) and the ligand molecule. Autodock 4.2 suite was used for molecular docking analysis and the docking logs were analyzed using the graphical user interface of ADT. Initially, the grid box was generated for the entire protein molecule, because the protein structure was not complexed with a small molecule. Further, at the end of the docking process (for each of the four protein), a possible ligand binding site was identified and another grid box was generated around that area. Then the final docking results in order to confirm the accuracy of the predicted binding sites. The results were clustered into similar conformations based on the cluster root mean square deviation and orientation. The dock score values are tabulated in Table: 3.

Visualization

Discovery studio 4.1 visualizer is a free, molecular modelling environment, for both small and macromolecule applications. It is developed by accelrys which specializes in scientific software products. It is used regularly in a range of academic and commercial entities, but is most relevant to pharmaceutical and biotechnology industries. The visualization of the docked compounds is tabulated in Table: 4

RESULTS

The targets identified for thymoquinone from *insilico* techniques are depicted in Table: 3

The Pharmacological activities of the *Nigella sativa*

Nigella sativa are antidiabetic, anticancer, immunomodulator, antimicrobial, anti-inflammatory, spasmolytic, bronchodilator, hepato-protective, renal protective, gastro-protective, antioxidant properties. The anti-cancer potency of *N.sativa* is predominantly contributed by its principle constituent thymoqui-

none. The targets were identified for thymoquinone by *Insilico* reverse screening process, it was observed that many of these identified targets (PDB-ID: 2XIR, 1NME, 1Q0R and IR9O), are significant contributor to the process of cell proliferation and apoptosis in cancer. Amino acid residues such as arginine, asparagine, lysine and leucine have bound to the thymoquinone compound. The compound showed good binding energy thus these compounds can be effectively used for the treating anti-cancer activity.

Table 1: Compounds and their Structure

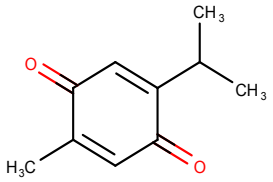
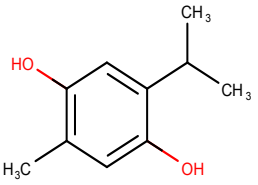
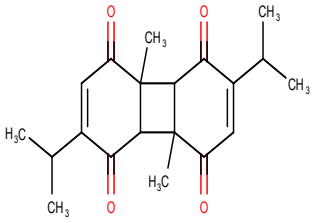
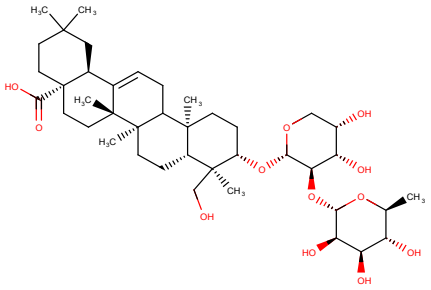
COMPOUNDS	STRUCTURES
THYMOQUINONE	
DIHYDRO THYMOQUINONE	
DITHYMOQUINONE	
ALPHA HEDERIN	

Table 2: Lipinski's Rule of Five

COMPOUND	MOL. WT	LOG P	HYDROGEN BOND DONOR	HYDROGEN BOND ACCEPTOR	MOLAR REFRACTIVITY
THYMOQUINONE	312.000000	-0.053101	5	6	77.145782

Table 3: Chemical Compound Thymoquinone and its Dock Score Values

COMPOUND	PROTIENS	BINDING ENERGY	HYDROGEN BOND CONTACT
THYMOQUINONE	2XLR	-5.78	Asn (900) H…D21 Leu (901) H…N
	1NME	-5.02	Asn (80) H…D21
	1Q0R	-6.25	Arg (179) H…H22
	IR9O	-6.2	Lys (48) H…N

CONCLUSION

The biologically active compounds of *N. sativa* are thymoquinone, alpha hederin, dithymoquinone, dihydrothymoquinone. From this study, TQ could be considered as an efficient phytochemical in regulating the protein responsible for cancer and thereby preventing the cancer initiation and development. This study will help to understand how the target protein is regulated by the ligands and inhibiting the carcinogenic pathway. In future studies, the identified targets have to be further validated in *in-vivo* and *in-vitro* bioassays to support the findings of this study.

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REFERENCES

1. Christine, M.K., and John, A.M., et al. The Role of Herbs and Spices in Cancer Prevention. J Nutr Biochem. 2008; 19(6): 347-361.
2. Houghton, P.J., Zarka, R., de las Heras, B., Hoult, J.R., et al. Fixed oil of Nigella sativa and derived thymoquinone inhibit eicosanoid generation in leukocytes and membrane lipid peroxidation. Planta Med. 1995; 61(1): 33-36.
3. Salomi, M.J., Panikkar, K.R., Kesavan, M., Donata, K., Rajagopalan, K., et al. Anti-cancer activity of nigella sativa. Anc Sci Life. 1989; 8: 262-266.
4. Aftab, A., Asif, H., Mohd, M., Shah, A.K., Abul, K.N., Firoz, A., et al. A review on therapeutic potential of Nigella sativa: A miracle herb. Asian Pac J Trop Biomed. 2013; 3(5): 337-352.
5. Amulyashree, S., Sadegh, S., Biplab, B., et al. Elucidation of molecular targets of bioactive principles of black cumin relevant to its anti-tumour functionality - An Insilico target fishing approach. Bioinformation. 2015; 10(11): 684-688.
6. Chehl, N., Chipitsyna, G., Gong, Q., Yeo, C.J., and Arafat, H.A. Anti-inflammatory effects of the Nigella sativa seed extract, thymoquinone, in pancreatic cancer cells. HPB (Oxford). 2009; 11(5): 373-381.
7. Tingfang, Yi., Sung-Gook, C., Zhengfang, Yi., Xiufeng, P., Melissa, R., Ying, W., Gautam, S., Bharat, B.A., and Mingyao, L., et al. Thymoquinone inhibits tumor angiogenesis and tumor growth through suppressing AKT and extracellular signal-regulated kinase signaling pathways. Mol Cancer Ther. 2008; 7: 1789-1796.
8. Gajendran, N., Aruldass, I., Dhanapal, S., et al. In silico docking studies on the anticancer effect of thymoquinone on interaction with phosphate and tensin homolog located on chromosome 10q23: A regulator of PI3K/AKT pathway. Asian Journal of Pharmaceutical and Clinical Research. 2015; 8(1): 192-195.