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INSILICO DOCKING STUDIES OF THYMOQUINONE AS POTENTIAL ANTI TUMOR DRUG TARGET

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ABSTRACT

Conventional cancer therapies cause serious side effects and, at best, merely extend the patient's lifespan by a few years. Cancer control may therefore benefit from the potential that resides in alternative therapies. Hence, in this insilico study, we have attempted to perform docking studies of thymoquinone and poloxime with apoptotic proteins. A Glide score of -10 or lower usually represent good binding.The results demonstrate that the probable targets if validated in wet lab may provide insight into deciphering the mechanism of action.

KEYWORDS: **Cancer, Thymoquinone,Poloxime, Apoptotic proteins,Bioinformatics, Schrodinger.**

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INTRODUCTION

Cancer is a disease characterized by uncontrollable cell growth.There are more than 100 different types of cancer, each is classified by the type of cell initially affected. The uncontrollable growth forms masses of tissue called tumors. According to the latest World Cancer Report from the World Health Organization (WHO), more women in India are being newly diagnosed with cancer annually. As against 4.77 lakh men, 5.37 lakh women were diagnosed with cancer in India in 2012. Breast cancer is also the most common cause of cancer death among women- 522,000 deaths in 2012. GLOBOCAN 2012 estimates predict a substantive increase of about 19.3 million new cancer cases per year by 2025, due to growth and ageing of the global population. More than half of all cancers (56.8%) and cancer deaths (64.9%) in 2012 occurred in less developed regions of the world, and these proportions will increase further by 2025. Conventional cancer therapies are known to cause serious side effects. Cancer control may therefore benefit from the potential that reside in alternative therapies. The demand to utilize alternative concepts or approaches in the treatment of cancer is therefore escalating. Plant molecules have shown significant potential in combating diseases through in vitro and in vivo studies. Around 25% of drugs used during the last 20 years are directly derived from plants, while the other 25% are chemically altered natural products. Still, only 5-15% of the approximately 250,000 higher plants have ever been investigated for bioactive compounds[1]. An ideal phytochemical is one that possesses anti-tumor properties with minimal toxicity and has a defined mechanism of action. One such source of curative solutions based on religious and cultural beliefs, to restore and sustain health is Black Seed, the name commonly used for the herbaceous plant, Nigella sativa L. Black seed is also identified as the curative black cumin in the Holy Bible, and is described as the Melanthion of Hippocrates and Dioscroides and as Gith by Pliny. It has also been mentioned by the Prophet of Islam for its benefits as being a healing for every disease [2,3].The focus in recent years has been on its ability to treat cancer by inhibiting cell growth & inducing apoptosis. Nigella sativa is showing encouraging chemo preventive as well as chemotherapeutic properties. The active components, specifically thymoquinone and dithymoquinone are thought to bring about this process. This plant is widely distributed in Southern Europe, Northern Africa and Southern Asia.In India it is found in Punjab, West Bengal, Sikkim, Bihar, Himachal Pradesh, Assam,Gangetic plains and Orissa[4].

Figure 1
Nigella Sativa seeds and flower

Chemical Structures Figure(1.1)

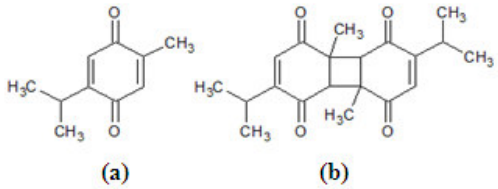


Figure 1.1
Chemical structures of thymoquinone (a) and dithymoquinone (b).

Figure 1.1(a) thymoquinone(TQ) is the main active constituent of the volatile oil of the black seed. Mahfouz and El-Dakhakhny were the first to report on the isolation of 'nigellone' from the oil of N. sativa seed, using Girard's reagent. TQ was later shown to be the main constituentof the volatile oil[5,6]. In addition, El-Dakhakhny determined that the 'nigellone' isolated had earlier been a dimer of TQ, which was later named dithymoquinone (TQ2) Figure 1.1(b). Dithymoquinone (TQ2) The latter compound was shown to be formed via photodimerization of TQ as a consequence of exposure to sunlight during separation and extraction of the Quinone's from the seed reported the isolation of Thymohydroquinone (THQ) from N. sativa seed volatile oil [7] .It has been reported that tumor formation has also resulted from decreased pro-apoptotic signals (p53), and increased anti-apoptotic signals (Bcl-2). While these oncogenes do exist in some types of cancer, it appears that the most influential factor leading to cancer is the loss of the tumor suppressor gene p53, when p53 function is lost it results in triggering cancer[8, 9].

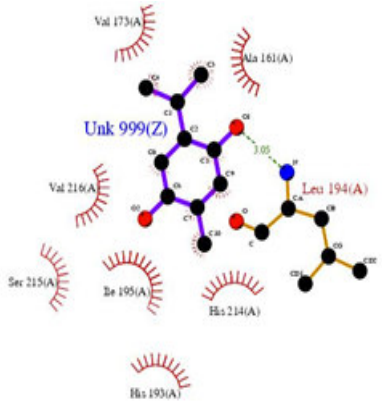
METHODOLOGY

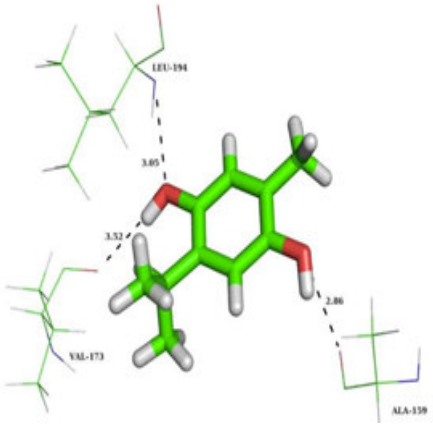
Thymoquinone (IMW) and Poloxime (PXE) structures were obtained from PDB. The target proteins for interaction analysis with IMW and PXE were taken from PDB in the pdb format. Docking protocols were used in order to predict the binding affinities of Thymoquinone and Poloxime .The docking scores and the binding affinity of these ligands was checked with selected proteins. Maestro - A powerful, molecular modeling environment by Schrodinger was used to provide an accurate and superior solutionfor design, selection and optimization of novel drug candidates was used so identify target proteins. It is vital in lead optimization to clearly understand the degree to which known binders or docking hits satisfy or violate complementarity to the receptor.

List of Proteins taken from PDB: PDB ID

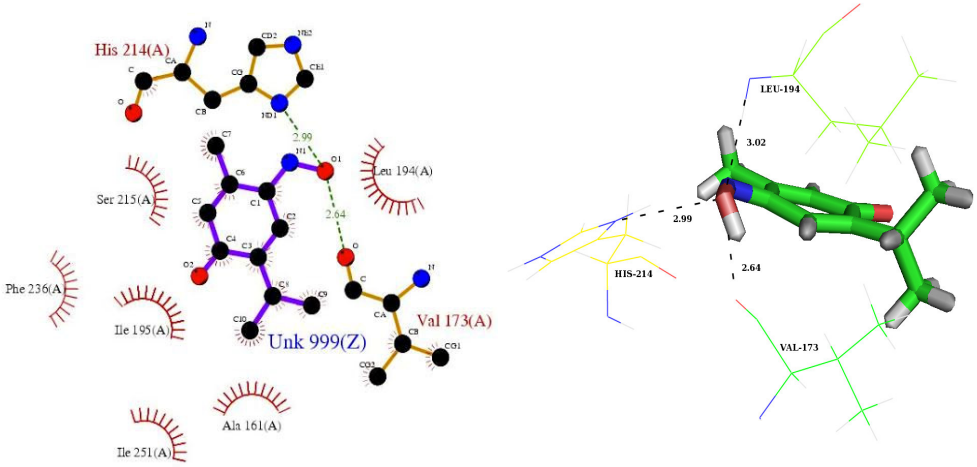
1. P53 -mutant 4MZI
2. HDAC 3SFH
3. PTEN 1D5R
4. Caspase3 3KJF

P53 (mutant)-4MZI with IMW

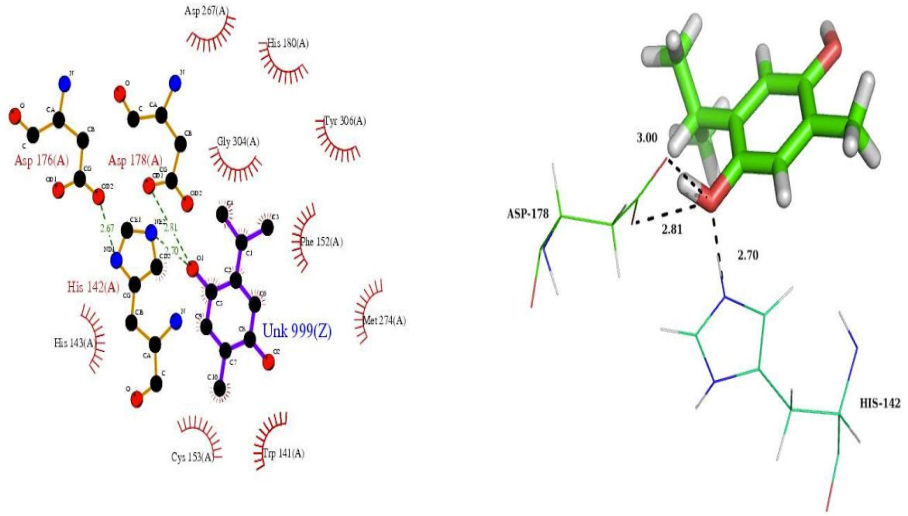




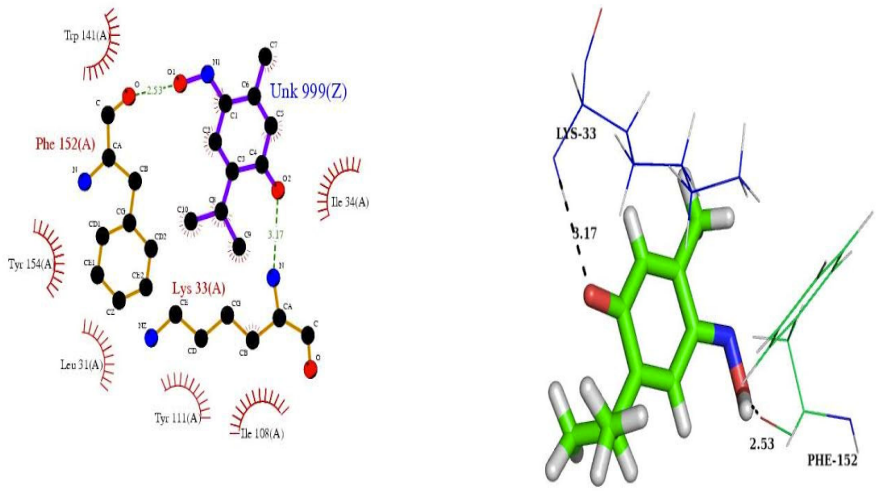
P53(mutant)-4MZI with PXE



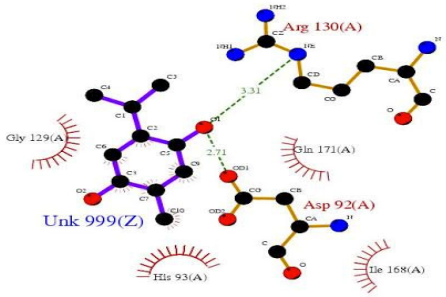
Histone deacetylase 3SFH_IMW

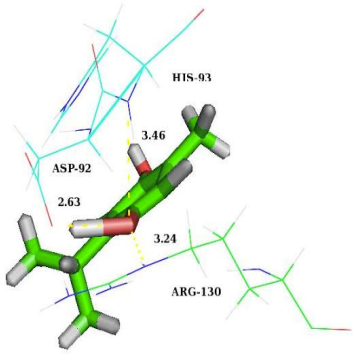


Histone deacetylase 3SFH_PXE

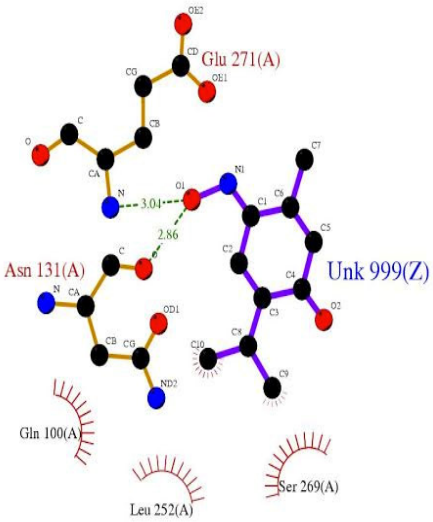


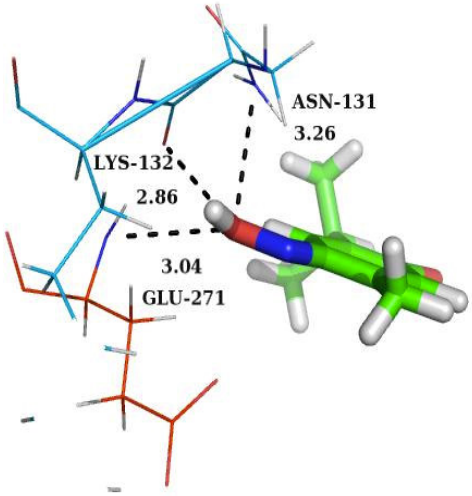
PTEN 1D5R_IMW



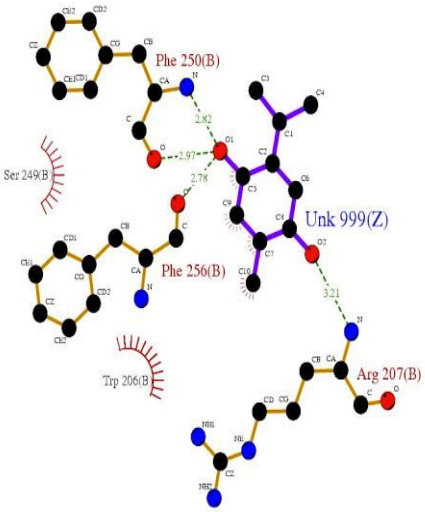


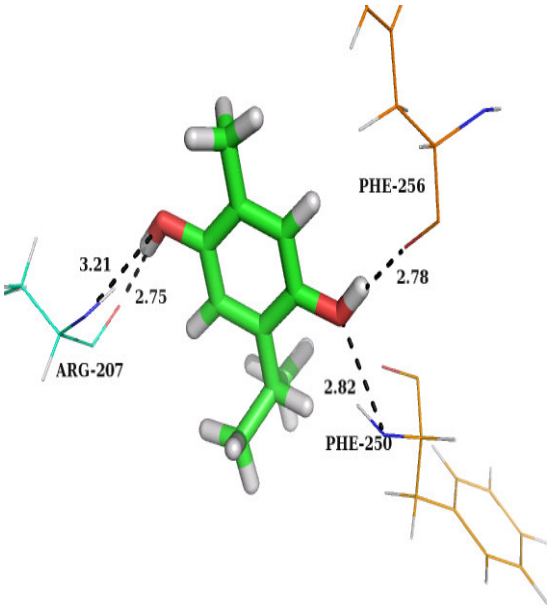
PTEN 1D5R_PXE



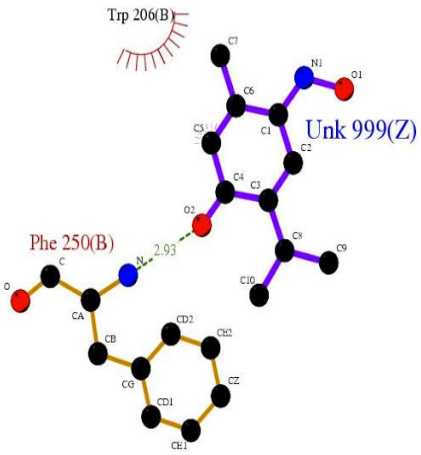


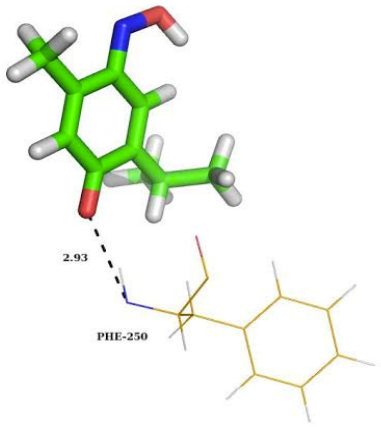
Caspase3 3KJF_IMW





Caspase3 3KJF_PXE





DISCUSSION

In guarding the cell against genetic damage, the p53 system functions both in the nucleus of the cell and in the cell's gel-like cytosol. When this system detects irreparable damage to the cell, p53 is unleashed to trigger apoptosis. In about half of all cancers, the p53 gene is rendered inoperable by mutation, enabling cancer cells to proliferate despite their genetic malfunctions. P53 Mutations in DNA-binding domain of TP53 gene may play role in the early onset and prognosis of breast cancer. Thus we tried to find the binding affinity of thymoquinone (IMW) and poloxime (PXE) to mutant p53 to see if it could bind to it and regularize its function , as research shows TQ induces apoptosis in cells by p53-dependent and p53-independent pathways[10]. HDAC inhibitors are a new class of drugs that interfere with the function of histone deacetylases, and are being studied as a treatment for cancer and neurodegenerative diseases. Finding out how thymoquinone functions as an HDAC inhibitor may help further this research[11]. Caspases play a central role in programmed cell death , thus are an attractive target for developing new therapeutics against cancer. We tested the binding affinity of IMW and PXE to caspase 3.Drug-induced apoptosis is associated with the activation of caspases increases in p53 expression[12,13]up-regulation of proapoptoticBax and downregulation of anti-apoptotic Bcl-2 and decrease in cyclins B1 and D1[14,15,]. The PTEN gene provides instructions for making a protein that is found in almost all tissues in the body. The protein acts as a tumor suppressor, which means that it helps regulate the cycle of cell division by keeping cells from growing and dividing too rapidly or in an uncontrolled way. The Glide energy for the docked proteins and ligands is less than -10 which means the scores are viable and merit further investigations

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Proteins	PDB ID	Ligands	Glide energy
P53 –mutant	4MZI	IMW	-26.380136
		PXE	-37.431790
HDAC	3SFH	IMW	-35.097113
		PXE	-36.674389
PTEN	1D5R	IMW	-20.486048
		PXE	-13.249749
Caspase	3KJF	IMW	-23.162164
		PXE	-25.867362

QIKPROP- RAPID ADME PREDICTIONS OF DRUG CANDIDATES

QikProp efficiently evaluates pharmaceutically relevant properties for over half a million compounds per hour, making it an indispensable lead generation and lead optimization tool. The Advantages of ADME PropertiesPrediction-Nearly 40% of drug candidates fail in clinical trials due to poor ADME (absorption, distribution, metabolism, and excretion) properties. The ability to detect problematic candidates early can dramatically reduce the amount of wasted time and resources, and streamline the overall development process. Accurate prediction of ADME properties prior to expensive experimental procedures, such as HTS, can eliminate unnecessary testing on compounds that will ultimately fail; ADME prediction can also be used to focus lead optimization efforts to enhance the desired properties of a given compound.QikProp for Thymoquinone and Poloxime was done to predict their ADME properties and was found to be acceptable according to the guideline values,which define that percentage of human oral absorption less than 25% is poor and higher than 80% is good. The percentage of human oral absorption according to QikProp for both Thymoquinone and Poloxime was found to be 81.792%.

CONCLUSION

Bioinformatics tools such as computer-based molecular docking were used to find the binding mechanism and probable molecular targets of Thymoquinone and Poloxime and thus their probable role in cell cycle arrest, repair and apoptosis of cells when required. Investigations in this line of study merits further evaluation.There may also be a need to find more modified forms or analogs of Thymoquinone to increase its binding affinity and efficacy and thus its viable therapeutic effect.

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