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Molecular Docking Studies of Phytoconstituents Identified in *Crocus sativus*, *Curcuma longa*, *Cassia occidentalis* and *Moringa oleifera* on Thymidylate Synthase – An Enzyme Target for Anti-Cancer Activity

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ABSTRACT

The aim and objective of the present study is to perform *in-silico* docking analysis of the major active constituents identified in four Indian medicinal plants namely *Crocus sativus*, *Curcuma longa*, *Cassia occidentalis* and *Moringa oleifera* for anti-cancer activity. *In-silico* docking analysis was performed by using Molegro Virtual Docker (MVD). The parameter used for the docking analysis are MolDock score, Rerank score and H-Bond interactions (binding energy). The target for anti-cancer activity is thymidylate synthase. The X-Ray crystal co-ordinate of thymidylate synthase (PDB ID- 1HVY) was retrieved from protein data bank in .pdb format. The phytoconstituents of four medicinal plants were retrieved from PubChem compound database in .mol format. The standard drugs Ralitrexed, 5-Fluorouracil and Vinblastine were obtained from the drug bank in .mol format for comparison. The comparative anti-cancer activity of the phytoconstituents of four medicinal plants are analysed by docking score and binding energy. It was analysed from the parameters of docking that the phytoconstituents from *Crocus sativum* showed better anti-cancer activity compared to that of the standard drugs.

INTRODUCTION

Cancer also known as a malignant tumor is a group of diseases involving abnormal cell growth with the potential to invade or spread to other parts of the body. Not all tumours are cancerous; benign tumors do not spread to other parts of the body. The number of new cancer cases ranges from 3.7 million in Eastern Asia to about 1800 in Micronesia/Polynesia.

Recently, The National Cancer Registry Programme (NCRP) commenced by the Indian Council of Medical Research, Bangalore, has published a report on Time Trends in Cancer Incidence Rates and it revealed that the total cancer cases are likely to go up from 979,786 cases in the year 2010 to 1,148,757 cases in the year 2020 (Takiar *et al.,* 2010). Thymidylate synthase is a key enzyme in the synthesis of 2'-deoxythymidine-5'-monophosphate, an essential precursor for DNA biosynthesis. The improved knowledge of the complex mechanism of the biological pathways in which thymidylate synthase is involved represents a unique chance to find new mechanism-based inhibitors, aimed to treat cancer. For this reason, this enzyme is a critical target in cancer chemotherapy. The most widely used inhibitor of thymidylate synthase is 5-Fluorouracil (5-FU) (Kawakami *et al.,* 2001). Recent years have witnessed a renewed interest in plants as pharmaceuticals in the Western world. Herbal medicines play a major role in primary health care, mainly in the developing countries. Therapeutic potential of herbal drugs are attributed to the bioactive phytochemical present in it. Plants are biosynthetic laboratories of a wide spectrum of chemicals of various physiological functions. These phytochemical are believed to have better compatibility with the human body and possess medicinal properties. Herbal drugs have a successful history as old as human civilization and today herbal medicines are coming back into prominence because of decreasing efficacy and serious side effects of the modern medicines (John *et al.,* 2012).

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Saffron is a spice derived from the flower of the saffron crocus (*Crocus sativus*) plant native to Southwest Asia. Significant information points to the ability of saffron to inhibit cancer (Abdullaev, 2003). Aqueous saffron preparations have been reported to inhibit chemically induced skin carcinogenesis (Das *et al.,* 2004). Both changes in carcinogen bio activation and tumour proliferation appear to occur (Das *et al.,* 2010). Curcumin (diferuloylmethane) is a polyphenol derived from the *Curcuma longa* plant, commonly known as turmeric. Curcumin, widely used as a spice and colouring agent in food, possesses potent antioxidant, anti-inflammatory and anti-tumour promoting activities (Kuo *et al.,* 1996). *Cassia occidentalis L*. is an annual or perennial ayurvedic plant which is used in several traditional medicines to cure various diseases. This weed has been known to possess antibacterial, antifungal, antidiabetic, anti-inflammatory, anticancerous, antimutagenic and hepatoprotective activity (Yadav *et al.,* 2010). *Moringa oleifera Lam* (Moringaceae) is a highly valued plant, distributed in many countries of the tropics and subtropics. It has an impressive range of medicinal uses with high nutritional value. In addition to its compelling water purifying powers and high nutritional value, *Moringa oleifera* is very important for its medicinal value (Anwar *et al.*, 2007).

The aim and objective of the present study is to perform *in-silico* docking analysis of the major active constituents identified in four Indian medicinal plants namely *Crocus sativus, Curcuma longa, Cassia occidentalis* and *Moringa oleifera* for anti-cancer activity on the enzyme target thymidylate synthase and compare the docking score and binding patterns with that of the standard drugs Ralitrexed, 5-Fluorouracil and Vinblastine.

MATERIALS and METHODS

Molecular docking studies

Preparation of Ligand

The major phytoconstituents are identified from the selected medicinal plants namely crocin, crocetin, picrocrocin from *Crocus sativus* (Escriban *et al.,* 1996), curcumin, DL-ar-turmerone, isagarin from *Curcuma longa* (Nutakul, 2013), pentalogin, scopoletin from *Cassia occidentalis* (Dholwani *et al.,* 2008) and niazimicin, niazirin from *Moringa oleifera* (Hussain *et al.,* 2014) which possess anti cancer properties according to traditional claims. The 3D structures of the active constituents are retrieved from PubChem chemical databases (Bolton *et al.,* 2008) and saved in .mol format. The ligands are imported to the workspace and preparation is done for docking studies. The docking scores of the active constituents are compared against the standard drugs (Ralitrexed, Vinblastine, 5- Fluorouracil) obtained from the drug bank in .mol format (Wishart *et al.,* 2008).

Preparation of Enzyme

The target for docking studies is selected as thymidylate syntahse. Docking analysis is done by initially selecting the target for the disease and followed by obtaining the 3D structure of thymidylate syntahse (1HVY) (Phan *et al.,* 2001) from protein data bank (Bernstein *et al.,* 1978) in .pdb format. It is well known that PDB files often have poor or missing assignments of explicit hydrogens, and the PDB file format cannot accommodate bond order information.

Therefore, proper bonds, bond orders, hybridization and charges were assigned using the MVD. The potential binding sites of both the targets were calculated using the built-in cavity detection algorithm implemented in MVD. The search space of the simulation exploited in the docking studies was studied as a subset region of 25.0 Angstroms around the active side cleft. The water molecules are also taken in to consideration and the replaceable water molecules were given a score of 0.50.

Molegro Virtual Docker's docking search algorithms and scoring functions

Ligand docking studies were performed by Molegro Virtual Docker (MVD), which has recently been introduced and gained attention among medicinal chemists. MVD is a fast and flexible docking program that gives the most likely conformation of ligand binding to a macromolecule. MolDock software is based on a new heuristic search algorithm that combines differential evolution with a cavity prediction algorithm (Thomsen and Christensen, 2006). It has an interactive optimization technique inspired by Darwinian Evolution Theory (Evolutionary Algorithms - EA), in which a population of individuals is exposed to competitive selection that weeds out poor solutions. Recombination and mutation are used to generate new solutions. The scoring function of MolDock is based on the Piecewise Linear Potential (PLP), which is a simplified potential whose parameters are fit to protein-ligand structures and a binding data scoring function (Gehlhaar *et al.,* 1998) that is further extended in GEMDOCK (Generic Evolutionary Method for molecular DOCK) (Yang and Chen, 2004) with a new hydrogen bonding term and charge schemes.

MolDock Optimizer

In MVD, selected parameters were used for the guided differential evolution algorithm: number of runs =5 by checking constrain poses to cavity option), population size=50, maximum interactions =2000, cross over rate=0.9, and scaling factor=0.5. A_o variance-based termination scheme was selected rather than root mean square deviation (RMSD).To ensure the most suitable binding mode in the binding cavity, Pose clustering was employed, which lead to multiple binding modes.

Parameters for scoring functions

MolDock score

They ignore-distant-atoms option was used to ignore atoms far away from the binding site. Additionally, hydrogen bond directionality was said to check whether hydrogen bonding between potential donors and acceptors can occur. The binding site on the protein was defined as extending in X, Y and Z directions around the selected cavity with a radius of 25 Angstroms.

Rerank Score

The reranking scoring functions are used to create and predict models for estimation of chemical properties (e.g. QSAR). The reranking score function is computationally more expensive than the scoring function used during the docking simulation but it is generally better than the docking score function at determining the best pose among several poses originating from the same ligand. While the rerank-score in MVD provides an estimate of the strength of the interaction, it is not calibrated in chemical units and it does not take complex contributions (such as entropy) into account. Even though the rerank score might be successful in ranking different poses of the same ligand, it might be less successful in ranking poses of different ligands. It is therefore recommend ranking the results of a virtual screening run using the rerank score. The binding affinity measure may then be used subsequently to get a rough estimate of the highest ranked poses.

DruLiTo Software

DruLiTo is open source software. It can calculate different molecular properties and screen the molecules based on drug likeness rules such as, 'The Lipinski rule of five' (Lipinski 2004), MDDR-like rule, Veber Rule, Ghose Filter, BBB Rule, CMC-50 like rule and Quantitative estimate of drug likeness (QED).

RESULTS and DISCUSSIONS

***In-silico* docking results**

The ability of the phytoconstituents to bind with the targets is given in terms of MolDock Score. The MolDock Score and re rank scoring are used as the parameters for analysing the docking results. The phytoconstituents are ranked according to their MolDock Score. The ligand possessing the highest mol dock and re rank score shows a strong affinity towards its target.

In-silico docking analysis of phytoconstituents from *Crocus sativus*, *Curcuma longa*, *Cassia occidentalis* and *Moringa oleifera* on thymidylate syntahse (PDB ID: 1HVY) ranking based on MolDock Score is represented in table 1 and HBond is represented in table 2. The binding pattern of crocetin are analysed using the ligand energy inspector tool built-in in the Molegro virtual docker. It was found that the binding patterns are similar to that of the standard drug Ralitrexed and it poses maximum MolDock Score as well as the rerank score. The structure 1HVY has in total 4 chains (A, B, C and D chains). Each chain of Thymidylate synthase possesses 288 residues. It was analysed that the chain C plays a major role in the binding to phytoconstituents including the standard drug.

The residues in the 1HVY chain C which are involved in the binding to the standard drug ralitrexed and crocetin are Ala 312, Arg 50, Arg 126, Arg 215, Asn 112, Asn 226, Asp 48, Asp 49, Asp 218, Asp 254, Cys 195, Gln 214, Glu 87, Glu 310, Gly 217, Gly 222, His 196, His 256, His 261, Ile 108, Leu 192, Leu 221, Lys 47, Lys 77, Lys 107, Lys 308, Met 311, Phe 80, Phe 225, Ser 216, Thr 51, Trp 109, Tyr 258, Arg 163, Arg 175, Arg 176, Asp 173, Asp174.The binding pattern for Ralitrexed, Crocetin, Curcumin and Niazimicin are represented in the figure 1,2,3 and 4 respectively.

The 'drug likeness properties' of the phytoconstituents was evaluated according to the 'The Lipinski rule of five' and to develop them as potential lead compound for anti-cancer activity. The rules, based on the 90-percentile values of the drug's property distributions, apply only to absorption by passive diffusion of compounds through cell membranes; compounds that are actively transported through cell membranes by transporter proteins are exceptions to the rule. The Lipinski criteria are widely used by medicinal chemists to predict not only the absorption of compounds, as Lipinski originally intended, but also overall drug-likeness. The results of the phytoconstituents analysed are represented in table 3.

All the phytoconstituents passes the drug likeness properties except Vinblastine and crocin.

Further studies can be performed to evaluate the *in-vitro* and *in-vivo* anticancer activity of the selected medicinal plants and to discover pharmacokinetic properties of the phytoconstituents to know the absorption, distribution, metabolism and excretion of the phytoconstituents.

Table 1: *In-silico* docking analysis of phytoconstituents from *Crocus sativus*, *Curcuma longa*, *Cassia occidentalis* and *Moringa oleifera* thymidylate synthase (PDB ID: 1HVY) ranking based on MolDock Score.

S. No.	Name	Ligand	MolDock Score	Rerank Score	HBond
1.	**[01]Ralitrexed**	**Ralitrexed**	**-173.887**	**-128.979**	**-11.2637**
2.	[00]Crocetin	Crocetin	-125.41	-105.362	-5.46598
3.	[01]Curcumin	Curcumin	-106.289	-82.4104	-3.746
4.	[00]Niazimicin	Niazimicin	-104.745	-94.2493	-6.91162
5.	[00]Picrocrocin	Picrocrocin	-101.803	-88.7085	-6.65758
6.	**[00]Vinblastine**	**Vinblastine**	**-91.9291**	**56.8852**	**-3.59816**
7.	[00]Niazirin	Niazirin	-89.8043	-88.3942	-6.41756
8.	[00]Turmerone	Turmerone	-89.0163	-73.2264	-0.219559
9.	[00]Scopoleptin	Scopoleptin	-77.1298	-66.3236	-4.2016
10.	[00]crocin	crocin	-65.728	-25.4591	-10.499
11.	**[00]Fluorouracil**	**Fluorouracil**	**-55.7535**	**-50.5361**	**-2.08843**
12.	[00]Pentalogin	Pentalogin	1971.95	44.9628	-0.442231

Table 2: *In-silico* docking analysis of phytoconstituents from *Crocus sativus, Curcuma longa, Cassia occidentalis* and *Moringa oleifera* thymidylate synthase (PDB ID: 1HVY) ranking based on HBond interaction.

S. No.	Name	Ligand	MolDock Score	Rerank Score	HBond
1.	**[01]Ralitrexed**	**Ralitrexed**	**-173.887**	**-128.979**	**-11.2637**
2.	[00]crocin	crocin	-65.728	-25.4591	-10.499
3.	[00]Niazimicin	Niazimicin	-104.745	-94.2493	-6.91162
4.	[00]Picrocrocin	Picrocrocin	-101.803	-88.7085	-6.65758
5.	[00]Niazirin	Niazirin	-89.8043	-88.3942	-6.41756
6.	[00]Crocetin	Crocetin	-125.41	-105.362	-5.46598
7.	[00]Scopoleptin	Scopoleptin	-77.1298	-66.3236	-4.2016
8.	[01]Curcumin	Curcumin	-106.289	-82.4104	-3.746
9.	**[00]Vinblastine**	**Vinblastine**	**-91.9291**	**56.8852**	**-3.59816**
10.	**[00]Fluorouracil**	**Fluorouracil**	**-55.7535**	**-50.5361**	**-2.08843**
11.	[00]Pentalogin	Pentalogin	1971.95	44.9628	-0.442231
12.	[00]Turmerone	Turmerone	-89.0163	-73.2264	-0.219559

Table 3: The Lipinski rule of five for evaluation of 'drug likeness properties' using DruLiTo Software.

Sr. No.	Title	MW	logP	HBA	HBD
1	Crocetin	328.17	4.472	4	2
2	Curcumin	368.13	1.945	6	2
3	Turmerone	216.15	3.669	1	0
4	Niazimicin	357.12	0.592	7	4
5	Pentalogin	285.21	2.723	2	1
6	Picrocrocin	330.17	-0.207	7	4
7	Scopoleptin	192.04	0.97	4	1
8	Fluorouracil	130.02	-1.137	4	2
9	Niazirin	269.13	0	6	4
10	**Vinblastine**	**753.98**	**3.056**	**13**	**0**
11	Ralitrexed	452.08	-1.314	4	3
12	**Crocin**	**976.38**	**-1.214**	**24**	**14**

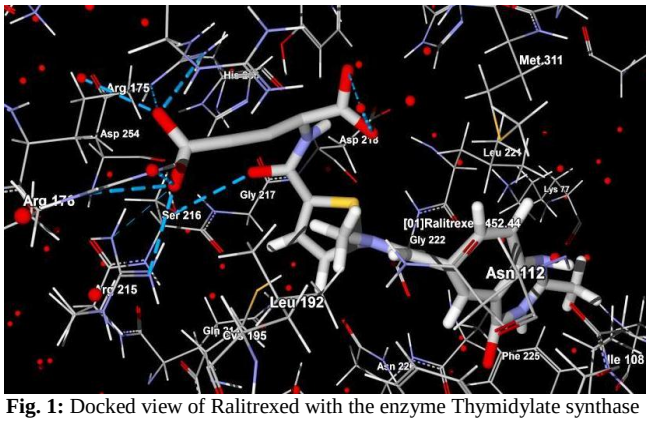

Fig. 1: Docked view of Ralitrexed with the enzyme Thymidylate synthase (1HVY)

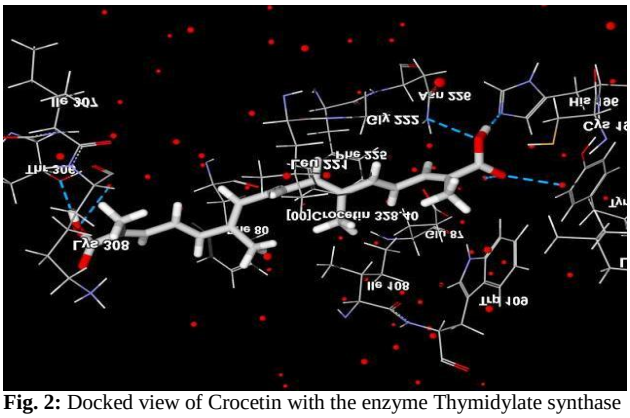

Fig. 2: Docked view of Crocetin with the enzyme Thymidylate synthase (1HVY)

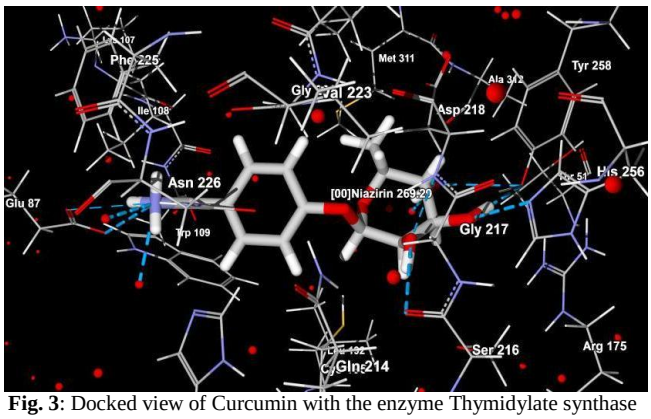

Fig. 3: Docked view of Curcumin with the enzyme Thymidylate synthase (1HVY)

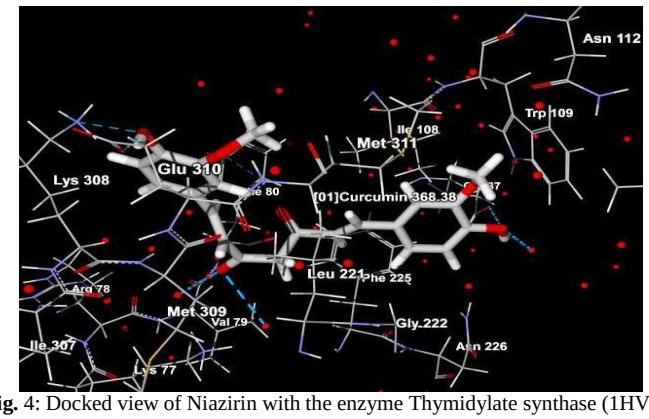

Fig. 4: Docked view of Niazirin with the enzyme Thymidylate synthase (1HVY)

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