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Molecular Approach Towards Screening of Biological Targets of Berberine and its Production Sources

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Abstracts

Traditional medicine dating back to centuries ago has been used to treat several ailments of humans. Berberine is a chemical compound that is found in plants which is being used in the form of drugs to work against various diseases. For optimum production of Berberine suitable plant species must be selected out of *Coptis japonica* and *Argemona mexicana* which are high yielding. From the pathway of Berberine production it is known that more the amount of substrate Canadine gives more amount of Berberine. The objective of this work is to compare the Canadine production efficiency among *Coptis japonica* and *Argemona mexicana* by molecular level studies for both the species. It is concluded that the enzyme, (S)-canadine synthase from *Argemona mexicana* is more efficient towards the production of (S)-canadine which in turn lead to Berberine production. Later the efficiency of Berberine against various disease targets is tested by molecular docking approach under same experimental conditions. Molecular docking results conferred the efficiency of Berberine in the treatment of gastric cancer over the other diseases.

Keywords: Berberine, Canadine, Canadine Synthase, Homology Modeling and Molecular Docking.

Introduction

Plants have been used for medicinal purposes long before prehistoric period. Chemical compounds extracted from plants have been known to counteract a number of ailments (1). Even though herbal medicines have lesser potent when compared to synthetic drugs in some cases, still they are considered lesser toxic or having lesser side effects in contrast to synthetic drugs. According to statistical data provided by World Health Organization (WHO), 80 percent of people worldwide rely on herbal medicines for some aspect of their primary health care needs. WHO also estimated that around 21,000 plant species have the potential for being used as medicinal plants for a wide range of diseases (2). Fast developing countries such as India and China are contributing as much as 80% of total natural sources for manufacturing of drugs, which are being used worldwide. According to the World Bank reports, trade in medicinal plants, botanical drug products and raw materials are growing at an impressive annual growth rate between 5 and 15%. In India the value of botanicals related trade is about US $10 billion per annum while the annual export of US is $1.1 billion. China's annual herbal drug production is worth a huge amount of US$48 billion with export of US $3.6 billion (3).

The observation is that drugs got from plants handled in unrefined structure without the segregation of active molecules would be safer (4). As the ultimate aim for any medicine is their non-toxicity, effectiveness, stability, specificity, and potency, herbal medicines are highly encouraged (5). Consistency in biological activity and composition are essential requirements for the safe and effective use of therapeutic drugs (3).

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Berberine is one of those herbal medicines found in several plants (6). Berberine (2, 3-methylenedioxy-9, 10-dimethoxy-protoberberine) is a chemical found in several plants which is a cure for a wide range of diseases. Berberine is a quaternary ammonium salt from the protoberberine group of benzylisoquinoline alkaloids (5). Benzylisoquinoline alkaloids (BIAs) are a large, diverse group of ~2500 specialized plant metabolites (2). Among them, the natural isoquinoline alkaloid Berberine has been employed in Ayurvedic and Chinese Medicine for hundreds of years with a wide range of pharmacological and biochemical effects (8). Berberine has a wide range of beneficial properties including anti-cancer, anti-inflammatory, anti-bacterial and cholesterol lowering effects. Berberine has shown impressive efficacy against various bacterial strains like cholera, giardia, shigella, and salmonella. It has the potential to work against even staphylococcus, streptococcus, and clostridium (9). Some of the most common side effects of Berberine usage include vomiting, hypertension (high blood pressure), nausea, chest pain, skin rashes, breathing problems and skin hives (10).

Berberine has been isolated from various plant families including Papaveraceae, Berberidaceae, Fumariaceae, Menispermaceae, Ranunculaceae, Rutaceae and Annonaceae (11). Berberine is found in significant amounts in *Coptidis rhizome, Coptis japonica. Coptis chinensis, Argemona Mexicana, Berberis aristate,*

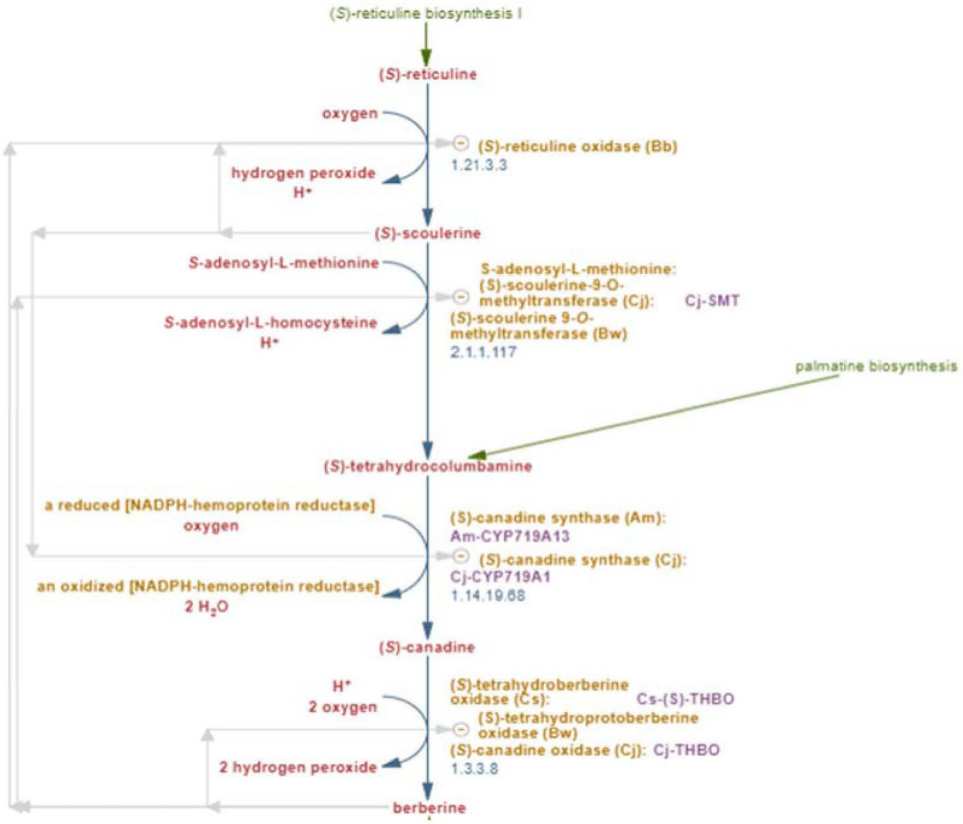

Fig. 1 : Berberine Biosynthesis Pathway

(Image courtesy: MetaCyc Pathway - Berberine biosynthesis)

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Berberis aquifolium, Berberis asiatica, Berberis croatica, Berberis thunbergii and *Berberis vulgaris* (9). Out of all the species producing Berberine, *Coptis japonica* and *Argemona mexicana* are the two species having considerable amount in them (12,13).

(S)-Canadine Synthase: Canadine is the last but one product in the pathway of production of Berberine (Figure 1). (S)-canadine synthase (EC 1.14.21.5) is an enzyme that catalyzes the chemical reaction:

(S)-Tetrahydrocolumbamine + NADPH + H^+ + O2 →(S)-canadine+ $NADP^+$ + 2 H_2O: Concludes that increase in the amount of Canadine leads to increase in Berberine production confirming Canadine as a non-substrate inhibitor (8). Hence this study is aimed towards the prediction of Canadine production efficiency from the two high yielding Berberine plant producers namely, *Coptis japonica* and *Argemona mexicana*.

Materials and Methods

Sequence and Structure Retrieval: Amino acid sequences of (S)-canadine synthase from the species *Coptis japonica* (Accession No: Q948Y1*)* and *Argemona Mexicana* (Accession No: B1NF19) are retrieved from NCBI in FASTA format. The 3D structure of Tetrahydrocolumbamine is taken from

Table 1: Targets of seven chosen diseases

S.No	Receptor	Receptor's PDB Code	Clinical Applications
1	Epidermal growthfactor receptor	1JL9	Breast cancer
2	Interleukin 6	1P9M	Skin aging
3	Wee1 like protein kinase	1X8B	Gastric cancer
4	BCL2 associated X protein	4ZGM	Gastric cancer
5	Acetyl-Co A carboxylase	4ASI	Hypercholesterolemia
6	ABCG2 Transporter	5DO7	Breast cancer

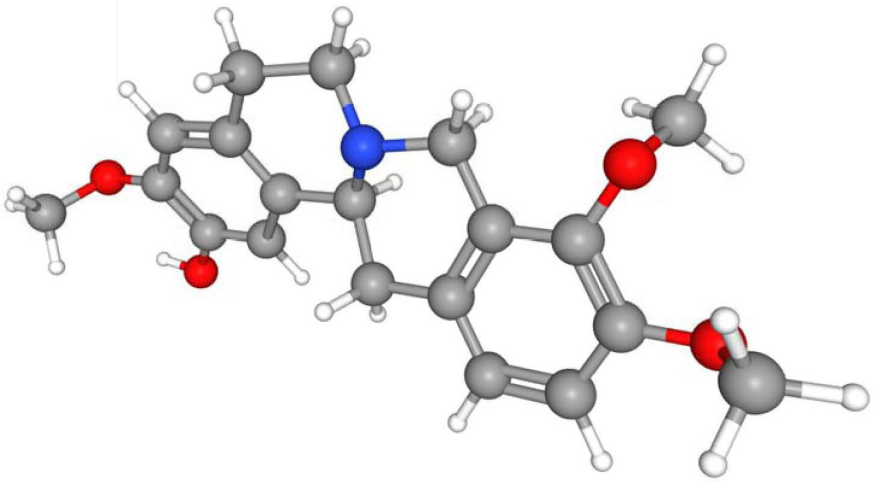

Fig. 2 : 3D structure of Tetrahydrocolumbamine

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PubChem database with the confirmer ID CID_440229 (figure 2). All the disease targets or receptors of various diseases like Breast cancer (1JL9, 5DO7), Skin aging (1P9M), Gastric cancer (1X8B, 4ZGM) and Hypercholesterolemia (4ASI) where the Berberine has proved its efficacy are retrieved from PDB (Table 1) (14).

Sequence Alignment and Structural Analysis of Coptis japonica and Argemona Mexicana : Local and global alignments between both the sequences is performed to find the sequence similarity pattern existing between them (15). This is followed by the primary and secondary structural analysis of (S)-canadine synthase from *Coptis japonica* and *Argemona Mexicana* to predict the physicochemical properties and the nature of secondary structural composition of the enzyme in both the plant species using ProtParam (16), SOPMA (17) and GOR4 (18) tools.

Structural Modelling and Validation: BLAST and PSI-BLAST were performed against PDB database for (S)-canadine synthase from *Coptis japonica* and *Argemona Mexicana* to find out the presence of any similar type of proteins whose structures are well studied.

Modelling of enzyme tertiary structures is done using I-TASSER [19] and PHYRE2[20] tools. The modelled enzyme structures from I-TASSER and PHYRE2 were validated by ProQ (21), PROCHECK (22), Verify3D [23], DFire Energy (24) and ERRAT (25) tools.

Energy Minimization and Molecular Docking: To proceed with the screening of best plant producer for Canadine which in turn gives Berberine among *Coptis japonica* and *Argemona Mexicana* based on molecular docking approach, the energy minimization of the modelled proteins and the substrate Tetrahydrocolumbamine is done using YASARA online web server tool (26). Berberine potency against various disease targets was studied by molecular docking approach using various tools like FRED (27), GOLD (28), Patch Dock (29), Surflex-Dock (30,31), AutoDock-Vina (32), Libdock and CDOCKER (33) etc., Unlike the above cases of docking performed in different experimental conditions using different tools where in the comparison of Berberine efficiency against the disease target becomes difficult, a total of six disease targets were allowed to interact with Berberine compound in an unified environment to easily analyze the potency of compound based on the dock score obtained. Energy minimization of the various disease receptors is done using UCSF chimera tool [34]. All molecular docking runs are executed in a flexible docking wizard called iGEMDOCK (35).

Results and Discussion

Structural Analysis of (S)-canadine synthase: Primary structural analysis of **(**S)-canadine synthase from *Coptis japonica* and *Argemona Mexicana* by PROTPARAM tool (Table 2) has confirmed both enzymes as positively charged, thermo stable and non-hydrophobic in nature based on the number of positive residues, aliphatic index and the GRAVY[a] scores they possess. However, **(**S)-canadine synthase from *Coptis japonica* was stable and the same enzyme from *Argemona Mexicana* was unstable based on their instability index scores.

Secondary structure analysis of (S)-canadine synthase from *Argemona mexicana* and *Coptis japonica* is performed using GOR4 and SOPMA tools (Table 3). This analysis revealed that, both the enzymes were rich in Alpha Helices and Random coils. They have a little Extended strand portion as their secondary structural element and the amount of Beta turns they possess was very little.

Sequence Alignment: In order to predict the similarity levels between the **(**S)-canadine synthase from *Coptis japonica* and *Argemona Mexicana* local and global sequence alignments were performed. This resulted into an identity score of 65.4% and 63.3% respectively (less than 70%). Therefore, both the amino acid sequences are sufficiently distinct for a chance of possessing different functionalities as enzymes.

Structural Modelling and Validation: As no experimental structures for (S)-canadine synthase

Table 2: PROTPARAM results for *Coptis japonica* and *Argemona mexicana*

PARAMETER	ORGANISM	
	Coptis japonica	***Argemona mexicana***
Number of amino acids	491	504
Molecular weight (g/mole)	55352.51	57452.49
Theoretical isoelectric point(pI)	8.69	9.14
Total number of negatively charged residues (Asp+Glu)	52	50
Total number of positively charged residues (Arg+Lys)	57	59
Instability index	37.06	44.74
Aliphatic index	96.17	93.23
GRAVY[a]	-0.053	-0.217

Table 3: GOR4 and SOPMA results for *Coptis japonica* and *Argemona Mexicana*

ORGANISM	SECONDARY STRUCTURE ANALYSIS TOOLS		
	PARAMETERS	**GOR 4**	**SOPMA**
Argemona mexicana	Alpha Helix	43.63%	45.63%
	Beta turn	0	4.17%
	Extended strand	11.51%	11.71%
	Random coil	42.86%	38.49%
Coptis japonica	Alpha helix	47.66%	47.66%
	Beta turn	0%	4.07%
	Extended strand	11.41%	9.98%
	Random coil	40.94%	38.29%

are available in biological databases till date, the enzyme's structure should be modelled by appropriate modelling tool. To choose the with the suitable modelling method, initially BLASTP search was performed to find the suitable templates with high level of sequence similarity. As PSI-BLAST is more sensitive than BLAST in finding distantly related sequences that are missed in a BLAST search, PSI-BLAST has also been performed to obtain accurate hits (36).

The percentage identity values of the first five hits obtained from BLASTp and PSI-BLAST of both the amino acid sequences of the (S)-canadine synthase from the species *Argemona mexicana* and *Coptis japonica* are all less than 30. As it resulted into very less percentage of sequence identity, where homology modelling approach is not suitable to proceed further, ab initio method is opted for tertiary structure modeling of the enzyme (S)-canadine synthase from Argemona Mexicana (Figure 3) and Coptis japonica (Figure 4) using I-TASSER and PHYRE2 tools.

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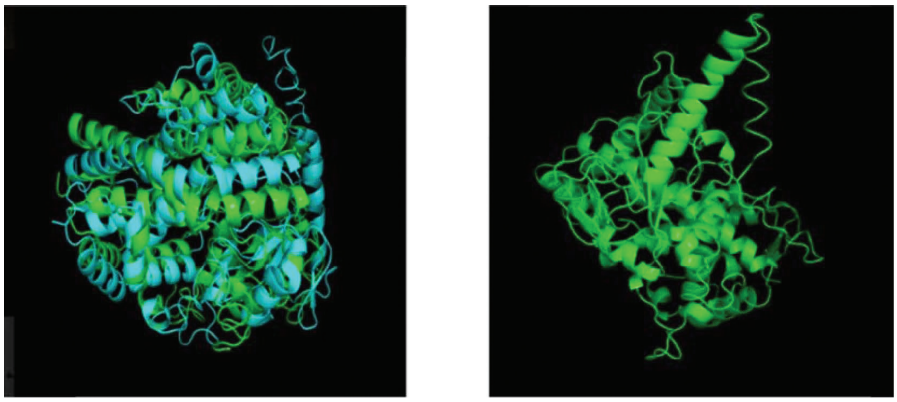

Fig. 3: Modeled structures of (S)-canadine synthase from *Argemona mexicana* using I-TASSER (a) and PHYRE2 (b) tools

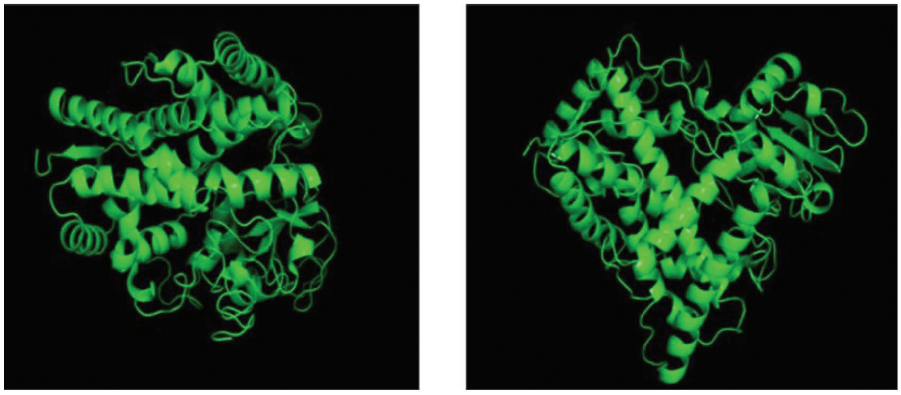

Fig. 4: Modeled structures of (S)-canadine synthase from *Coptis japonica* using I-TASSER (a) and PHYRE2 (b) tools

The modeled structures of the enzyme (S)-canadine synthase from *Argemona mexicana* and *Coptis japonica* from both I-TASSER and PHYRE2 are validated using tools like ERRAT, ProQ, Verify 3D, DFire Energy (http://www.scfbio-iitd.res.in/software/proteomics/protsav.jsp) (Table 4).

Based on results of all the above validation tools I-TASSER models were concluded as the best models for enzyme (S)-canadine synthase from *Argemona mexicana* and *Coptis japonica.* Hence, the modelled enzyme structures from I-TASSER tool were revalidated through Ramachandran plot analysis using Procheck (Table 5) tool. Having more than 97% of the residues in the favored and allowed regions both the I-TASSER modelled structures proved their reliability for the further use.

Molecular Docking: Stable and flexible docking has been performed using iGEMDOCK between the energy minimized structures of Tetrahydrocolumbamine and ab initio modelled (S)-canadine synthase from *Argemona mexicana* and *Coptis japonica* as ligand and receptors respectively (table 6).

Table 4 : Comparison of PHYRE2 and I-TASSER structures

Model	***Coptis japonica***		***Argemona mexicana***		
Validation	**PHYRE 2**	**I-TASSER**	**PHYRE 2**	**I-TASSER**	**Remarks**
Tools					CORRECT : LG score > 1.5 Max Sub > 0.1
ProQ	LG score: 4.548 Max Sub: 0.103	LG score: 6.263 Max Sub: 0.209	LG score: 4.005 Max Sub: 0.117	LG score: 6.162 Max Sub: 0.211	GOOD: LG score > 3 Max Sub > 0.5 VERY GOOD: LG score > 5 Max Sub > 0.8
Verify 3D	132.5601	156.0701	120.09	178.6701	Higher the quality score, higher the quality
DFire Energy	-978.98	-1116.54	-1087.27	-1171.88	Lower the energy, better the quality of structure
ERRAT	51.1161	79.7101	68.145	86.492	Good resolution- 95% or higherlower resolutions- around 91%

Table 5: Ramachandran plot parameters for I-TASSER structures

Procheck	***Coptis japonica***	***Argemona mexicana***
Number of residues in favored region	72.7%	72.1%
Number of residues in allowed region	25%	25.2%
Number of residues in outlier region	2.3%	2.8%

Table 6 : Fitness values from iGEMDOCK

LIGAND	**RECEPTOR ((S)-canadine synthase)**	**Docking Score**
Tetrahydrocoloumbamine	*Argemona mexicana* *Coptis japonica*	-102 kcal/mole -98.9431 kcal/mole

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The best docked poses are obtained between the modeled enzymes along with respective fitness values (Figure 5). Lower the free energy better is the binding between the ligand and receptor. As the free energy of *Argemona mexicana*'s (S)-canadine synthase against Tetrahy- drocoloumbamine is lower than that of *Coptis japonica*, it can be concluded that the enzyme, (S)-canadine synthase from *Argemona mexicana* is more efficient.

Further proceeding with determining the Berberine efficiency as a therapeutic agent against various diseases (37-45), stable docking is performed for all the six disease targets individually against Berberine as ligand, under same experimental conditions (Figure 6, Table 7).

From the above tabulated values, the docking energy for 1X8B (-115.301 kcal/mole) is the least. 1X8B is the disease target for gastric cancer. Based on the docking results, out of all the six disease targets, the Wee1 like protein kinase is efficiently blocked by Berberine. The inhibition of Wee1 like protein kinase leads to premature mitosis and cell death. Berberine may act as an effective therapeutic drug for the treatment of gastric cancer. Therefore, Berberine is most efficient in acting against this disease target when compared to others.

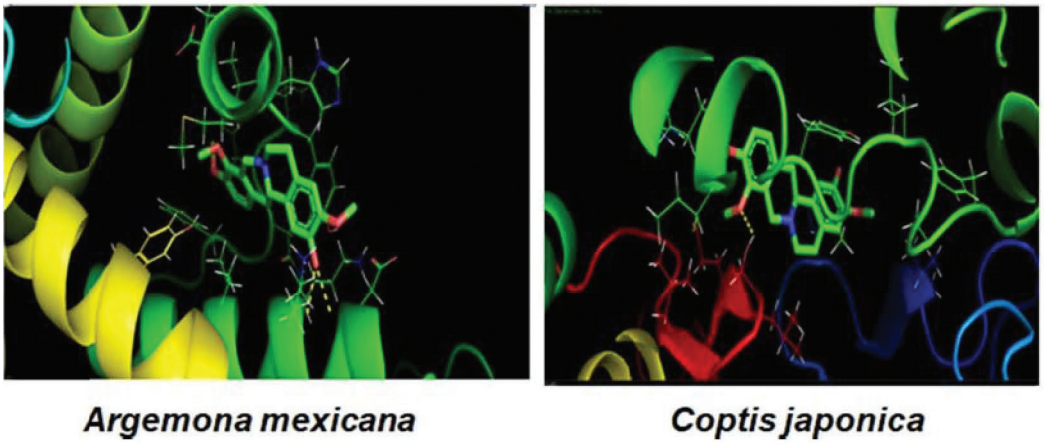

Fig. 5: Interaction of Tetrahydro-columbamine with modeled structures

Table 7: Docking energy values for six receptors against Berberine

S.No	Receptor	iGEMDOCK Docking energy (kcal/mole)
1	1JL9	-83.05
2	1P9M	-105.48
3	1X8B	-115.301
4	4ZGM	-89.3
5	4ASI	-102.35
6	5DO7	-94.3

Conclusion

Coptis japonica and *Argemona mexicana* are the two Berberine high yielding plant species. (S) canadine synthase is an enzyme that catalyzes the reaction of Canadine production from which Berberine is synthesized. Global and local alignments between the enzyme sequences from both the species confirmed that they are distant from each other. The structural analysis confirmed that both the species are producing the same kind of enzyme with most of the similar characteristics. Molecular docking of the validated and modelled

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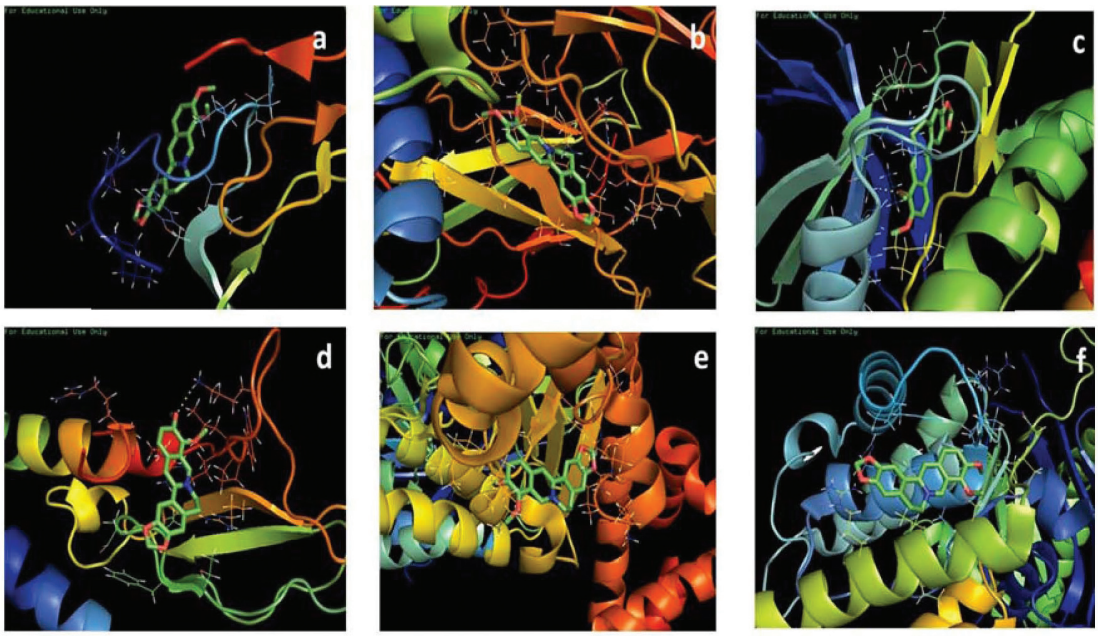

Fig. 6 : Best docked pose of Berberine against 1JL9 (a), 1P9M (b), 1X8B (c), 4ZGM (d), 4ASI (e) and 5DO7 (f)

structures with Tetrahydrocolumbamine has proved *Argemona mexicana* as a potent Canadine producer over *Coptis japonica* which in turn produce Berberine, with the energy value of -102 kcal/mole. The subsequent docking runs of Berberine against with its disease targets confirmed the higher therapeutic capacity of Berberine in treatment of Gastric Cancer over the breast cancer, skin ageing and hypercholesterolemia.

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