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AN *IN-SILICO* APPROACH FOR THE IDENTIFICATION OF ANTI-COLON CANCER PHYTOCOMPOUNDS FROM *AVENA SATIVA.L*

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ABSTRACT

Oats (*Avena sativa* L.) have been used as livestock and human foods since ancient times. It is a good source of many active phytocompounds which exhibit anti-inflammatory, anti-oxidant and anti-colon cancer activities. In the present study, an *in-silico* approach was followed to identify the potential phytocompounds of oats against anticolon cancer. Fourteen phytocompounds of oats were selected as ligands and subjected to molecular docking analyses for the inhibition of c-Met receptor which is the potential stimulator for colon cancer. The 3D structure of ligands and receptor were retrieved using online tools Pubchem and PDB, respectively. The structure of ligand molecules were drawn using chemsketch software. Out of 14 phytocompounds, 10 compounds satisfied the Lipinski's properties and the docking studies for these 10 compounds were done using commercial tool Accelyrs Discovery Studio 2.1. Among 10 compounds, Gallic acid showed the highest dock score i.e 126.44 with more hydrogen bond formation i.e 9.0. The results implied that Gallic acid will act against human colon cancer by blocking c-Met receptor and its can be developed into a potent drug for human colon cancer in future.

Key words: **Oats, Phytocompounds, *In-Silico* approach, Gallic acid.**

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INTRODUCTION

For a long period of time, plants have been a valuable source of natural products for maintaining human health, with more intensive studies for therapies[1]. The medicinal value of the plants lies in some phytocompounds that will produce a definite physiological action on the human body. The most important bioactive constituents of the plants are alkaloids, tannins, flavanoids and phenolic compounds[2]. *Avena sativa L.* is commonly known as oats, the seeds of this plant have been used as both human and animal food source due to their high nutritional content. The seeds containing high levels of proteins, lipids, vitamins, polyphenols, dietary fibers, and minerals[3]. The polyphenols of oats have shown properties such as anti-HIV[4], anti-oxidants[5], anti-diabetes[6], anti-inflammatory, anti-itching[7], anti-colon cancer[8] and wound healing[3]. Cancer is a major public health burden in both developed and developing countries. Colorectal cancer, is a cancer from uncontrolled cell growth in the colon or rectum[9]. Symptoms of the colon cancer include rectal bleeding and anaemia which are sometimes associated with weight loss and changes in bowel habits. Colon cancer occurs due to lifestyle and increasing age with only a minority of cases associated with underlying genetic disorders. The limited success of clinical treatment including radiation, chemotherapy, immune modulation and surgery in treating cancer, as evident by the high morbidity and mortality rates indicates that there is on imperative need of new cancer management[10]. Consumption of oats and oat bran will reduce the risk of colon cancer because of their high fibre content and also due to phenolic compounds which will stop the proliferation of colonic cancer cells[8]. Hepatocyte growth factor (HGF) is a scatter factor and its receptor c-Met is a proto-oncogene play important roles in tumor formation and progression[11]. *In-Silico* analysis, a computational method helps to study the formation of intermolecular complexes as well as to speed up the drug discovery. Drug activity can be determined through molecular binding of the ligand to the receptor / protein. In their binding conformation, both the molecules exhibit geometric and chemical complementarity. These two characters are very most important for successful drug activity. The computational approach to search for a ligand that is able to fit both geometrically and energetically in the binding site of the receptor is called *in-silico* or molecular docking analysis[12]. The present study is aimed to perform a docking analysis using bioactive compounds of oats into the c-Met receptor (potential stimulator for tumor formation) to determine the probable binding model for anti- human colon cancer[13].

MATERIALS AND METHODS

Phytocompounds screened

Fourteen phytocompounds namely avenanthramide, avenanthramide A, avenbenzoic acid, caffeic acid, vanillic acid, vanillin, ferulic acid, sinapic acid, syringic acid, p-coumaric acid, anthranilic acid, p-hydroxyl benzoic acid, cinnamic acid, gallic acid were retrieved from available HPLC data of oats[14].

Preparation of protein structure
Protein Data Bank (PDB)

The PDB is the global archive for information about the 3D structure of bio-macromolecules and their complexes[15]. c-Met selected as a receptor in the present study based on the literature and the 3D structure of the receptor was downloaded from Protein data bank (http://www.rcsb.org/pdb/home.do) with the specific resolution and the PDB ID is 3CIX.

Preparation of ligand structures
ChemSketch

ACD/Chemsketch is the powerful chemical drawing and graphics package from ACD/Labs software, which will draw molecular structures, reactions and calculate chemical properties very quickly and easily. The three dimensional structures of phytocompounds were drawn by Chemsketch[16].

Lipinski's analysis

Lipinski's rule says that to evaluate drug likeness and determine the pharmacological activity. The Lipinski's properties like molecular weight, log p, number of hydrogen bond donors and acceptors. Lipinski's parameters to satisfy the retrieved fourteen phytocompounds of oats were analysed, using PubChem tool[17].

Docking the inhibitors against the active site of the receptors

Docking is a computational technique which is used to determine the interaction between ligand and the receptor based on dockscore. The hydrogen bond length, number of hydrogen bonds and scoring functions are used to assess the conformations of the probable protein binding site. The inhibitor and target protein was geometrically optimized and docked using docking engine Accelrys Discovery Studio (2.1)[18].

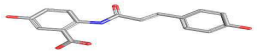
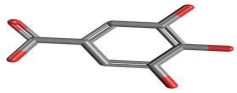
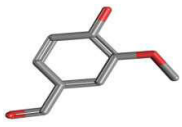
Accelrys Discovery Studio (2.1)

Accelrys Discovery Studio (2.1) is a life science modelling and simulation application focused on optimizing the drug discovery process. The mechanism for ligand placement is based on the fitting points. Fitting points are added to hydrogen bonding groups on the protein and ligand. A molecular mechanics like scoring function and number of hydrogen bonds is employed by dock score to rank the docked posses[19].

RESULTS AND DISCUSION

Molecular docking study shows that the inhibitory pathway of the potential drug target against colon cancer using bioinformatics tools[20]. In the present investigation, the oat grains was selected to identify its potential bioactive compounds against colon cancer. c-Met receptor considered to play an important role in inhibiting the colon cancer activities and found as the most active compound in the respective target site. Out of 14 bioactive compounds, 10 compounds satisfied the Lipinski's properties. The selected ligands and c-Met receptor were subjected to docking studies using commercial tool Accelyrs Discovery Studio 2.1. Among 10 compounds, gallic acid showed highest dock score i.e. 126.44 and good hydrogen bond interaction. Gallic acid may be the promising candidate for inhibiting the c-Met receptor which is one of the stimulators for induction of colon cancer[13]. According to Christopher A. Lipinski's rule five is important for drug development where a pharmacologically active phytocompound should have not more than 5 hydrogen bond donors, not more than 10 hydrogen bond acceptors, molecular weight under 500 dalton, Partition coefficient A Log P less than 5. In the present study fourteen phytocompounds of oats were collected from available HPLC data and the 3D structure of the phytocompounds were retrieved and evaluated for Lipinski's properties using pubchem. Out of 14 compounds 10 compounds namely avenanthramide A, caffeic acid, ferulic acid, gallic acid, p-coumaric acid, p- hydroxyl benzoic acid, sinapic acid, syringic acid, vanillic acid and vanillin satisfied the Lipinki's properties (Table-1).

Table 1
Lipinski's properties of the ten compounds of oats

Ligand molecule	Molecular weight [g/mol]	Molecular Formula	Xlogp3 value (<=5)	H-bond donor	H-bond acceptor	Structure
Avenanthramide A	299.27812	$C_7H_6O_2$	1.9	1	2	
Caffeic acid	180.15742	$C_9H_8O_4$	1.2	3	4	
Ferulic acid	194.184	$C_{10}H_{10}O_4$	1.5	2	4	
Gallic acid	170.11954	$C_7H_6O_5$	0.7	4	5	
p-Coumaric acid	164.15802	$C_9H_8O_3$	1.5	2	3	
p- Hydroxyl benzoic acid	138.12	$C_7H_6O_3$	1.58	3	4	
Sinapic acid	224.20998	$C_{11}H_{12}O_5$	1.5	2	5	
Syringic acid	198.1727	$C_9H_{10}O_5$	1	2	5	
Vanillic acid	168.14672	$C_8H_8O_4$	1.4	2	4	
Vanillin	152.147	$C_8H_8O_3$	1.2	1	3	

According to Trapani *et al*. (1992) , a single hydrogen bond would not be expected to support a drug-receptor interaction alone, but when multiple hydrogen bonds, high dock score value and low bond length are formed between drugs and receptor, a significant amount of stability is conferred upon the drug-receptor interaction. Similarly in the present investigation, the interaction between Gallic acid and c-Met receptor conferred a significant amount of stability when compared to other compounds (Table-2). Gallic acid showed high dock score value i.e. 126 .44 and totally it produced nine hydrogen bond interactions with

the residues ARG1170 (4 hydrogen bonds), GLU31172 (4 hydrogen bonds) and GLY1280 (1 hydrogen bond) of the receptor whereas other compounds produced low dock score value which will minimize the interaction between ligand and the receptor.

Table 2
Molecular Docking analysis for the identification of bioactive compounds against colon cancer

Ligand molecule	Amino acid	Atoms in amino acid	Position	Atoms in ligand	H-bond length (Å)	H-bond	Dock score
Avenanthramide A	ARG	HH12	1170	O4	1.05	5	33.015
	ARG	HH21	1170	O2	2.48		
	GLU	OE1	1172	H25	1.76		
	GLU	OE2	1172	H25	2.46		
	TYR	O	1356	H35	1.21		
Caffeic acid	ARG	O	1279	H19	1.82	4	82.833
	THR	O	1356	H20	2.37		
	GLU	OE1	1172	H21	0.32		
	GLU	OE2	1172	H21	2.31		
Ferulic acid	ARG	HH21	1170	O4	2.33	4	45.425
	ARG	HH22	1170	O4	2.10		
	GLU	OE1	1170	H23	0.18		
	GLU	OE2	1170	H23	2.34		
Gallic acid	**ARG**	HE	1170	O3	2.37	9	126.44
	ARG	HH11	1170	O4	2.47		
	ARG	HH21	1170	O3	2.49		
	GLU	OE1	1172	H15	0.33		
	GLU	OE2	1172	H15	2.19		
	GLU	OE1	1172	H16	0.49		
	GLU	OE2	1172	H16	2.17		
	ARG	O	1170	H17	1.63		
	GLY	O	1280	H18	2.27		
p-coumaric acid	ARG	HH21	1170	O3	2.49	4	56.275
	ASN	HN	1353	O1	1.85		
	THR	O	1278	H19	1.56		
	GLU	OE1	1172	H20	0.80		
p-hydroxy benzoic acid	ARG	HH22	1170	O1	1.83	4	62.705
	ASN	HN	1358	O1	1.86		
	GLU	OE1	1172	H16	1.71		
	GLU	OE2	1172	H16	2.42		
Sinapic acid	ARG	HH12	1170	O3	2.9	5	29.344
	ARG	HH22	1170	O1	1.70		
	GLU	HN	1172	O4	2.30		
	ASN	HN	1358	O1	1.11		
	GLU	OE1	1172	H28	1.85		
Syringic acid	ARG	HH12	1170	O3	2.27	5	69.645
	ARG	HH22	1170	O1	1.84		
	THR	O	1356	H17	2.30		
	GLU	OE1	1172	H24	0.29		
	GLU	OE2	1172	H24	0.30		
Vanillic acid	ARG	HH12	1170	O4	2.08	3	98.805
	GLU	OE1	1172	H19	0.28		
	GLU	OE2	1172	H19	2,44		
Vanillin	ARG	HH21	1170	O1	2.19	3	48.444
	ARG	HH22	1170	O1	2.43		
	GLU	OE1	1172	H19	0.59		

Analysis of docking interaction with c-Met receptor crystal structure (3CIX) was performed to identify the original binding mode between the ligands. Crucial interaction between the ligands (green colour) and target protein c-Met (red colour) were shown in Figure 1-10. The yellow ring in the figures indicates the number of hydrogen bonds formed between ligands and receptor during molecular docking analysis. The c-Met residues interacting with the ligands were shown in stick model. Based on the results of molecular docking, the gallic acid showed much better binding energy with c-Met receptor. The result thus

identifying a strong potential phenolic compound i.e gallic acid as a better inhibitor for c-Met receptor

Figure 1-10
Interaction of drug-receptor complexes

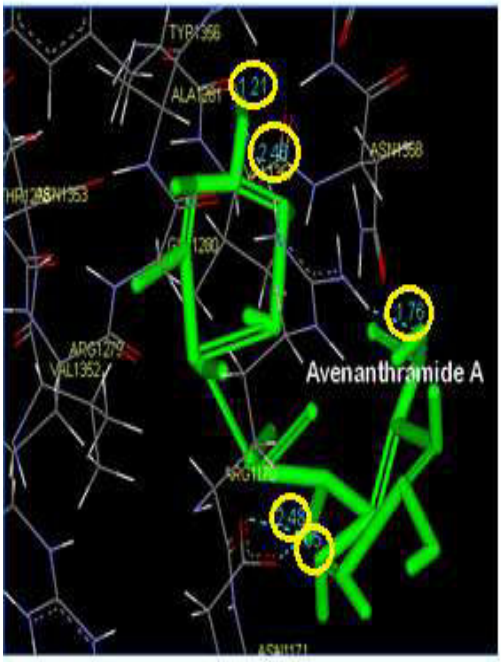


Fig-1 Avenanthramide A

Fig- 2 Caffeic acid

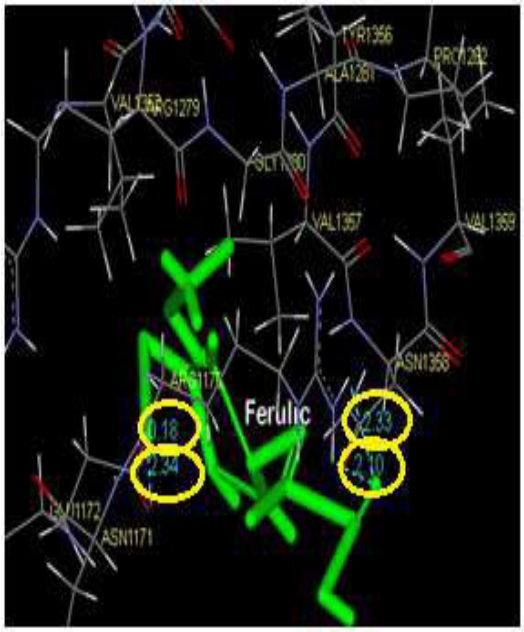


Fig- 3 Ferulic acid

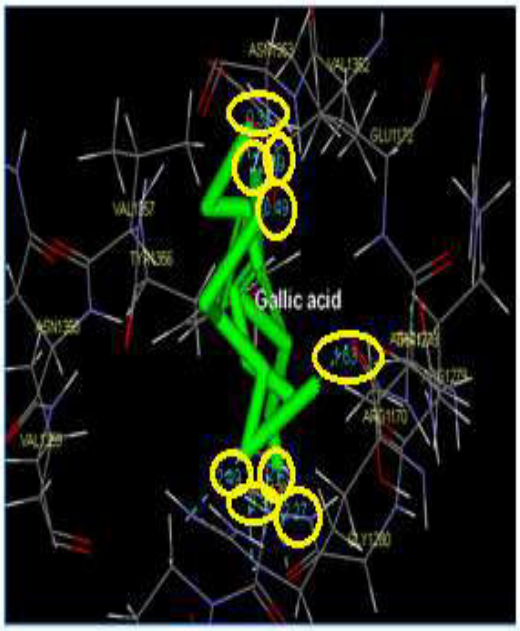


Fig- 4 Gallic acid

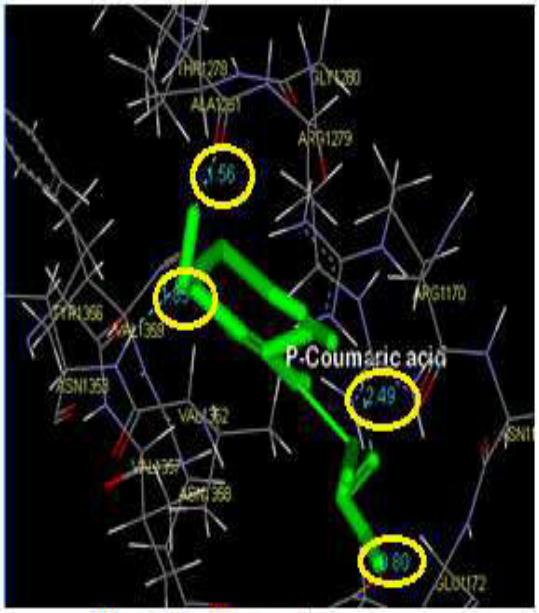


Fig- 5 P- Coumaric acid

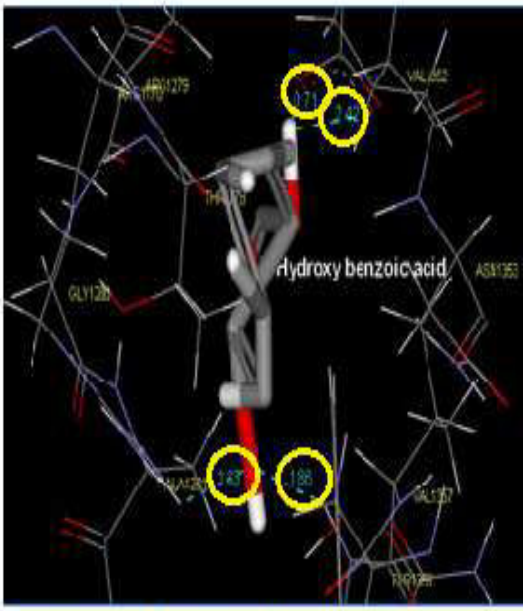


Fig-6 P-Hydroxy benzoic acid

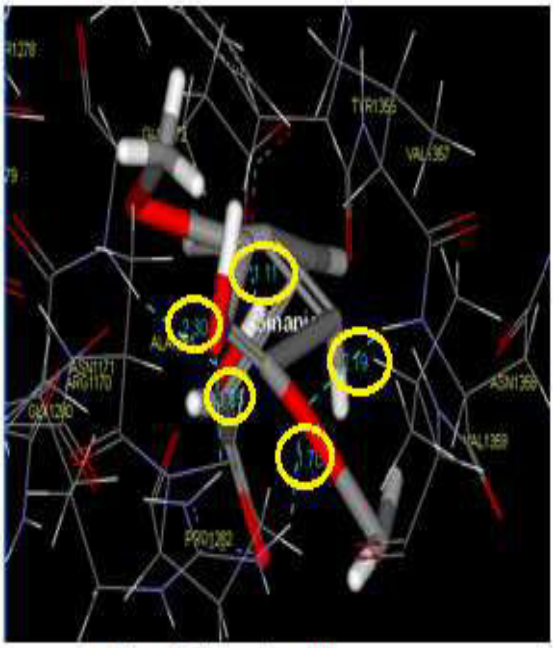


Fig- 7 Sinapic acid

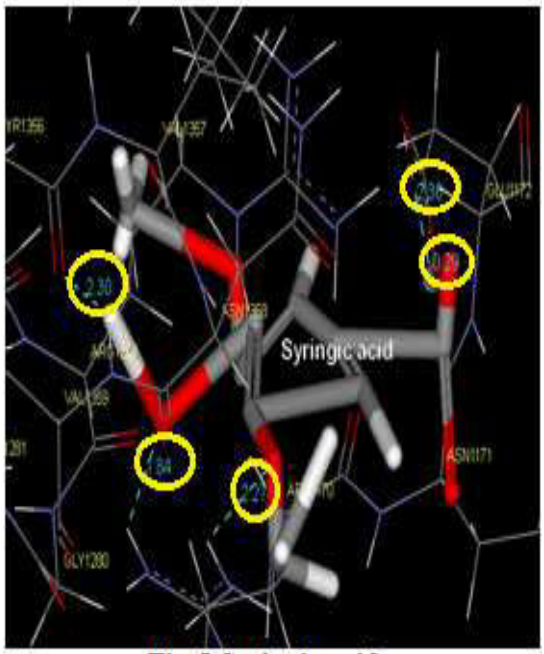


Fig-8 Syringic acid

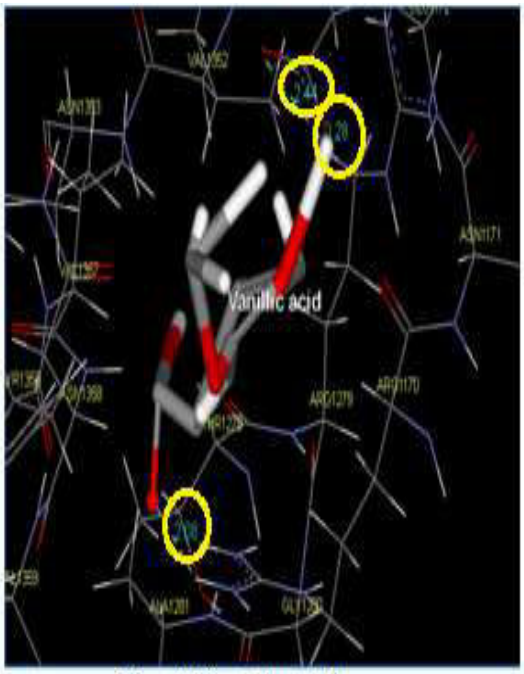


Fig- 9 Vanillic acid

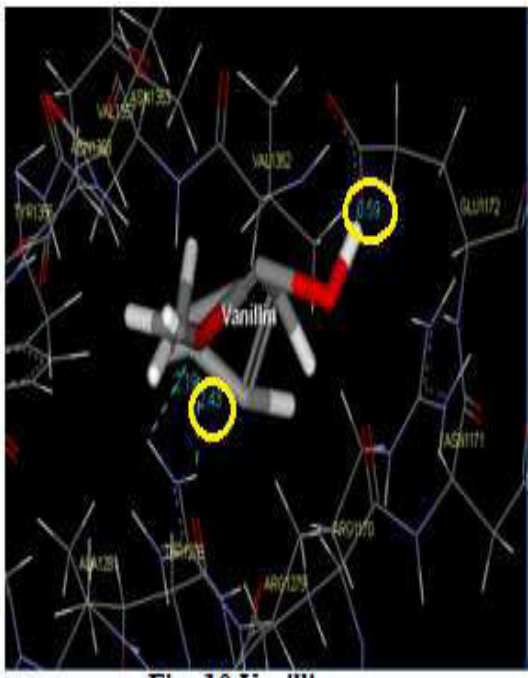


Fig- 10 Vanillin

CONCLUSION

The protein-ligand interaction plays a significant role in structural based drug designing and it is used to reduce cost and time in drug discovery[23]. In the present study docking simulation was performed to find out the binding orientation and binding affinities of the ten bioactive compounds and c-Met receptor using Accelrys Discovery Studio (2.1). Identifying the location of ligand binding sites on a protein, *de novo* drug design and structural identification and comparison of functional sites were the fundamental importance in molecular docking[24]. In the present investigation, out of 14 compounds 10 compounds showed interaction between ligand and the receptor. Out of 10 compounds the only one bioactive compounds of oats i.e gallic acid holds more promising drug formation against colon cancer based on docking analysis. Further *in-vivo* and *in-vitro* approaches are required to elucidate the molecular mechanisms of this compound to act as potent drug against colon cancer.

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