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INSILICO IDENTIFICATION OF MOLECULAR TARGETS FOR AJOENE [GARLIC COMPONENT] AND THEIR BINDING MODE ANALYSIS

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ABSTRACT

Identification of molecular targets for chemicals derived from plant origin may help in understanding the therapeutic potential of many unknown phytochemicals. Ajoene, a component of garlic is known to have various therapeutic potential such as anti-thrombotic, anti-inflammatory, anti-cancer, and anti-proliferative and also anti-microbial mainly with anti-fungal and anti-parasitic property. This study was carried out in quest to discover established and novel targets for ajoene using 'insilico reverse screening' technique and check their binding properties with ajoene. This was performed with the help of two pharmacophore screening servers PharmMapper and PharmaGist. The study resulted in identification of 59 molecular targets that found to have highest affinity to ajoene which confirms the therapeutic properties of ajoene. The study has also characterized new set of molecular targets which can be validated with invivo and invitro studies and also to analyze a set of targets for any plant based chemical component having biological significance.

KEY WORDS

Ajoene, binding mode analysis, garlic component, inverse screening, molecular targets,

INTRODUCTION

Non-conventional herbal medicines play a key role in the cure of several diseases from ancient times [1]. The synergistic effects of their phytochemicals help in the management of disease. Conventionally various nutraceuticals have shown to be anti-cancer, anti-diabetic, anti-inflammatory, anti-microbial in nature. Many spices, fruits, vegetables have shown to possess immunoprotecive, cardioprotective, neuroprotective abilities. Thus the identification of the targets by *invivo* or *invitro* or *insilico* methods for these nutraceuticals is gaining a prime importance.

Ajoene is a sulfur rich compound of garlic, formed mainly from pure allicin **Fig 1.**

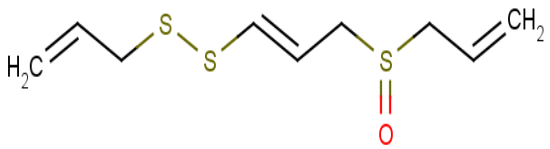

Fig 1: Structure of Ajoene

It is chemically more stable than allicin [2]. Several studies have proved the therapeutic importance of ajoene to be anti-thrombotic, anti-cancer, and anti-proliferative in action [3,4,5]. It is also found to be anti-microbial mainly with anti-fungal and anti-parasitic property [6,7,8]. A study on its anti-oxidant properties

has disclosed that ajoene activates protein kinase C delta-dependent Nrf2 activation, a potent target for oxidative stress found in many diseases [9]. In another study it has exerted potent effects on cell survival, apoptosis and adipogenesis in 3T3-L1 adipocytes [10]. A work on anti-diabetic effect of ajoene has suppressed the plasma glycemic and triglyceride levels thus highlighting its importance in clinical studies [11]. Ajoene lowered the levels of thiobarbituric acid reactive substance (TBARS) along with increased rate of glutathione peroxidase (GSH-Px), superoxide dismutase (SOD) and catalase (CAT) in the serum of stroke stage of SHRSP thus acting as anti-hypertensive agent [12]. The proposed mechanism for its anti-tumor action include induction of cell cycle arrest at G2/M phase, apoptosis of dividing cells and anti-adhesion properties against cancer cell lines [13,5].

The projected methods for anti-inflammatory property of ajoene are either by inhibiting COX-2 pathway by increasing LPS-induced COX-2 protein [14] or reducing the expression of iNOS [15]. It also possess anti-mutagenic properties by hindering mutagenesis induced by 2 mutagens benzo[a]pyrene (B[a]P) and 4-nitro-1,2-phenylenediamine (NPD) in a dose-dependent manner [16]. In various *in vivo* works it has demonstrated anti-cardiovascular ability either by preventing thrombosis[17], inhibiting cholesterol biosynthesis or anti-platelet effects [18].

Though many molecular targets are identified for ajoene, there still exists a mystery regarding its other molecular targets and the probable mechanism for its therapeutic effects. In the present work we have characterized its pharmacological targets through 'insilico reverse screening' approach using PharmMapper and PharmaGist followed by docking studies. The molecular targets obtained were classified in to several categories based on their therapeutic index. The docking studies for all the targets (nearly 100) were conducted to validate the results obtained by Pharm Mapper.

MATERIALS AND METHODS

Screening of Molecular Targets for ajoene

The 3d structure file of ajoene was extracted from PubChem (CID 5386591) and was submitted to the PharmMapper server [http://59.78.96.61/pharmmapper/]. This server screens the putative molecular targets for given small molecule using pharmacophore mapping strategy providing targets, their PDB IDs, function and fit score. This server is backed up by in house repertoire of annotated pharmcophore Pharm Target DB extracted from several other specialized databases like Target Bank, Binding DB, PDTD and Drug Bank. It conducts an automatic search to obtain the best mapping pose for the submitted query molecule against its own data base. The output gives N best fit and their aligned poses for each target. The 300 possible targets were selected on the basis of fit score and classified into anti-thrombotic, anti-coagulant, anti-inflammatory, anti-apoptotic, signal transduction modulators, epigenetic etc. based on the functions of the targets.

Target Screening

The redundant targets with different PDB IDs were further screened with the help of PharmaGist [http://bioinfo3d.cs.tau.ac.il/pharma/index.html].

This uses ligand-based pharmacophore detection. It aligns a set of drug-like molecule that can bind to the receptors and pharmacophore detection of ligand. Ajoene was taken as a pivot molecule to build pharmacophore of native ligand and ajoene. The result set consists of candidate pharmacophores computed by multiple flexible alignments of the input ligand. Hence this server was used to align the ajoene and targets bound native ligand and the best alignment was taken as a condition to screen the targets. The screening of the targets was done by comparing the molecular surface similarity of ajoene and the target molecules. The ligand files of targets and ajoene were submitted to the PharmaGist (only targets molecules which had repeated entry in PharmMapper) in .Mol2 file format. While submitting, the molecule was set as 'first input molecule' under a set a key-molecule in an advanced option and a min number of pharmacophore feature was set to 5. The ligand which showed either good alignment with ajoene or highest score was chosen and only its target was taken for further analysis. This search yielded 90 probable targets.

Binding Site

PDBsum (http://www.ebi.ac.uk/pdbsum/) database was used to find the native ligand binding site for the

given receptor. The output of PDBsum is a colour, or black-and-white, PostScript file giving a simple and informative representation of the intermolecular interactions and their strengths, including hydrogen bonds, hydrophobic interactions and atom accessibilities. The native ligand interactions of PDB protein and its dimensions were used to set the AutoGrid map during AutoDock process. The LigPlot interactions map of 90 targets were retrieved from this database and used for docking.

Analysis of Binding Mode using AutoDock

AutoDock 4.2 was used for the docking study combined with the Lamarckian genetic algorithm (LGA) to search for the globally optimized conformation. The 90 targets obtained after screening in PharmaGist were docked. During each docking experiment, 50 runs were carried out. Other parameters used for docking was grid map size 60× 60 × 62, grid point and the centre was calculated as per different receptors. At the end of a docking experiment with multiple runs, a cluster analysis was performed. Docking solutions with a ligand all-atom root mean square deviation (RMSD) within 0.1 nm of each other were clustered together and ranked by the lowest docking energy. Docked structures were ranked based on the binding energy. The binding energy as well as binding site residue of the receptor for ajoene and native ligand was considered for screening the final targets.

3.RESULTS AND DISCUSSION

In the present study, molecular targets for ajoene were identified using insilico technique and target-ajoene binding modes were analyzed by Autodock 4.2. And following were the results obtained.

3.1 Potential Targets for Ajoene

The number of potential targets identified for ajoene using pharmacophore target screening strategy with PharmMapper followed by pharmacophore detection of ligand with the help of PharmaGist yielded 90 targets. The classified list of the targets based on their function and the disease involvement are listed in the **Supplementary Table 1.**

Supplementary Table 1: The table lists the targets which were found similar to ajoene. In total 90 out of 300 targets were shortlisted to carry out further analysis.

Sl. No	Therapeutic Category	Name of Receptor targeting	Pharm Mapper Rank	PDB ID	PDB Ligand ID
1	Anti-thrombotic Targets	Prothrombin	174	1H8I	PHV
2	Anti-atherosclerotic Targets	Oxysterols receptor LXR-beta	246	1UPV	444
3		Oxysterols receptor LXR-alpha	106	1UHL	444
4	Anti-coagulant Targets	Endothelial protein C receptor	116	1L8J	NAG
5		cGMP-specific 3,5-cyclic phosphodiesterase	193	1UHO	VDN
6		Coagulation factor X	300	2UWO	701
7	Anti-hypercholesteromicTargets	3-hydroxy-3-methylglutaryl-coenzyme A reductase	277	2R4F	RIE
8		Bile acid receptor	75	3BEJ	MUF
9		Fatty acid-binding protein, adipocyte	113	1TOU	B1V
10		Lanosterol synthase	257	1W6K	LAN
11		Glycogen synthase kinase-3 beta	56	1Q3D	STU
12	Anti-hypertensive Targets	Neprilysin	186	1R1J	OIR
13		Renin	177	2V12	C39
14		Phenylalanine-4-hydroxylase	111	1KW0	H4B
15	Anti-obesity Targets	Corticosteroid 11-beta-dehydrogenase isozyme 1	171	3BEL	POX
16	Other Important Targets for CVD	Stromelysin-1 (MMP3)	153	1D8F	SPI
17		Estradiol 17-beta-dehydrogenase 1	85	1I5R	HYC
18	Anti-depressive Targets	Amine oxidase [flavin-containing] B	119	2BK3	FOH

19	Anti-diabetic Targets	Aldose reductase	212	1X98	FIS
20		Glucokinase	94	1V4S	MRK
21	Anti-inflammatory Targets	Cathepsin K	13	1TU6	FSP
22		Mitogen-activated protein kinase 14	244	2GFS	PQB
23		Leukocyte elastase	32	1B0F	SEI
24		cAMP-specific 3,5-cyclic phosphodiesterase 4B	36	1XMU	ROF
25		Carbonic anhydrase 2	33	1I90	INM
26		Mitogen-activated protein kinase 10	49	3FV8	JK3
27		Sulfotransferase family cytosolic 2B member 1	294	1Q22	AND
28		ADAM 17	123	1ZXC	IH6
29		Serum albumin	215	1GNI	OLA
30		Glucocorticoid receptor	198	1P93	DEX
31		Methionine aminopeptidase 2	168	1R5G	AO1
32		Mineralocorticoid receptor	241	2AA6	STR
33		Cathepsin B	180	1GMY	APD
34		Leukotriene A-4 hydrolase	299	1GW6	BES
35		Macrophage metalloelastase	263	1UTZ	PF3
36		Dual specificity mitogen-activated protein kinase kinase 1	22	1S9J	BBM
37		Caspase-1	135	1BMQ	MNO
38	Signal Transducing Modulators	Tyrosine-protein phosphatase non-receptor type 1	156	1NWL	964
39		cAMP-specific 3,5-cyclic phosphodiesterase 4D	114	1XON	PIL
40		Peptidyl-prolyl cis-trans isomerase FKBP1A	138	1BL4	AP1
41		cAMP-dependent protein kinase catalytic subunit alpha	47	1XH5	R68
42		Heat shock protein HSP 90-alpha	127	2VCJ	2EQ
43		Proto-oncogene tyrosine-protein kinase ABL1	196	2F4J	VX6
44		Serine/threonine-protein phosphatase PP1-gamma catalytic subunit	289	1JK7	OKA
45		Nicotinamide mononucleotide adenylyltransferase 1	275	1GZU	NMN
46		Angiopoietin-1 receptor	15	2P4I	MR9
47		Phosphoenolpyruvate carboxykinase, cytosolic [GTP]	34	1M51	TSX
48		Receptor tyrosine-protein kinase erbB-4	42	3BBT	FMM
49		GTPase HRas	78	1P2S	GNP
50		Cytochrome P450 2C8	255	1PQ2	PLM
51	Other Important Targtes for Cancer	Proto-oncogene tyrosine-protein kinase LCK	89	2OG8	1N8
52		Proto-oncogene tyrosine-protein kinase Src	150	1Y57	MPZ
53		Deoxycytidine kinase	216	1P62	GEO
54	Anti-apoptotic Targets	B-Raf proto-oncogene serine/threonine-protein kinase	227	1UWH	BAX
55	Antioxidant Targets	Aldo-keto reductase family 1 member C3	221	1S2A	IMN
56		Glutathione S-transferase P	217	2PGT	GPR
57		Glutathione S-transferase A1	242	1GUH	GSB
58		Aldo-keto reductase family 1 member C2	50	1IHI	IU5

59		Aldo-keto reductase family 1 member C1	259	1MRQ	STR
60		Alpha-tocopherol transfer protein	63	1R5L	VIV
61		Glutathione-requiring prostaglandin D synthase	279	2VD1	D28
62	Cell Cycle Targets	Cell division protein kinase 2	178	1OIQ	HDU
63		Kinesin-like protein KIF11	283	2UYM	K03
64		Cyclin-A2	211	2C5V	CK4
65	Nuclear Receptors	Nuclear receptor subfamily 1 group I member 2	98	1ILH	SRL
66		Retinoic acid receptor gamma	90	1FCZ	156
67		Hepatocyte growth factor receptor	272	1R0P	KSA
68		Thyroid hormone receptor beta	167	2J4A	OEF
69		Vascular endothelial growth factor receptor 2	248	2OH4	PTR
70		Retinoic acid receptor RXR-alpha	195	1FBY	REA
71		Progesterone receptor	258	1E3K	R18
72		Retinoic acid receptor RXR-beta	271	1H9U	LG2
73		Nuclear receptor ROR-alpha	218	1S0X	C3S
74		Epidermal growth factor receptor	233	1XKK	FMM
75		Estrogen receptor beta	254	1NDE	MON
76		Mast/stem cell growth factor receptor	124	1T46	STI
77		Retinoic acid receptor beta	169	1XAP	TTB
78		Vitamin D3 receptor	91	1S19	MC9
79		Basic fibroblast growth factor receptor 1	118	2FGI	PD1
80		Estrogen receptor	234	1YIN	CM3
81		Androgen receptor	25	1GS4	ZK5
82		Peroxisome proliferator-activated receptor gamma	235	1RDT	570
83	Other Important Biological Targets	Beta-secretase 1	120	2IQG	F2I
84		Retinol-binding protein 4	286	1QAB	RTL
85		Sex hormone-binding globulin	160	1LHO	AOM
86		Histo-blood group ABO system transferase	11	1R7U	DLG
87		S-methyl-5-thioadenosine phosphorylase	290	1SD2	MTH
88		Serine hydroxymethyltransferase, cytosolic	295	1BJ4	PLP
89		Bile salt sulfotransferase	102	1OV4	AE2
90		Transthyretin	52	1RLB	REA

Supplementary Table 2: The table lists the molecules which were docked with ajoene, the binding energy of ajoene and native ligand. Also the electrostatic energy and inhibition constant are also tabulated.

Sl No.	Target name	PDB ID	Binding site		Docking		Binding energy		Electrostatic energy		Inhibition constant	
			Amino acid	atom	Native ligand	Ajoene	Native ligand	Ajoene	Native ligand	Ajoene	Native ligand	Ajoene
	Anti- apoptic Target											
1	B-Raf proto-oncogene serine/threonine-protein kinase	1UWH	Glu500-A	OE2	-	y	-5.13	-	-0.07	-	173.64	
			Asp593-A	N	y	-	-	-6.94	-	-0.07		8.21
	Anti-coagulant Target											
2	Endothelial protein C receptor	1L8J	ASN 30- A	ND2	y	y	-3.73	-4.65	0.02	0	1.84	393
	Anti-diabeticTargets											
3	Glucokinase	1V4S	ARG A 63	N	y	y	-5.51	-9.2	-0.07	-0.11	91.96	181.83
4	Aldose reductase	1X98	Trp A 20	NE1	y	y	-6.67	-9.07	-0.06	-0.09	12.82	223.09
	Anti-hypercholesteromic Targets											
5	Fatty acid-binding protein, adipocyte	1TOU	Tyr 128(A)	OH	y	y	-4.16	-6.27	-0.15	-0.15	420.39	25.5
			Arg 126(A)	NH2	y	y	-4.16	-6.27	-0.15	-0.15	420.39	25.5
6	3-hydroxy-3-methylglutaryl-coenzyme A reductase	2R4F	Arg 590(A)	NH1	y	y	-4.12	-7.87	-0.41	-2.42	820.88	1.7
			Ser 684(A)	OG	y	y	-4.12	-7.87	-0.41	-2.42	820.88	1.7
			Lys 735(B)	NZ	y	y	-4.12	-7.87	-0.41	-2.42	820.88	1.7
			Lys 691(A)	NZ	y	y	-4.12	-7.87	-0.41	-2.42	820.88	1.7
	Anti-hypertensive Targets											
7	Phenylalanine-4-hydroxylase	1KW0	Leu 249(A)	O	y	y	-5.9	-7.86	-0.08	1.55	47.32	1.75
8	Neprilysin	1R1J	Arg 717(A)	NH2	y	y	-4.97	-7.9	-0.1	-0.81	228.4	1.61
	Anti-inflammatory Targets											
9	Leukocyte elastase	1BOF	Ser 195(A)	N	y	y	-5.04	-5.4	-0.1	-0.08	493.64	253.99
10	Caspase-1	1BMQ	Arg 341(B)	NH1	y	y	-4.28	-8.44	-0.27	-0.24	647.4	730.9
11	Cathepsin B	1GMY	Gln 23(A)	NE2	y	y	-4.37	-4.51	-0.13	-0.83	626.54	493.47
12	Serum albumin	1GNI	Arg 117(A)	NH2	y	y	-4.71	-5.07	-0.24	-1.41	351.44	193.2

13	Leukotriene A-4 hydrolase	1GW6	His 295(A)	NE2	y	y	-5.48	-6.23	0.2	-2.09	95.47	23.9
			His 299(A)	NE2	y	y	-5.48	-6.23	0.2	-2.09	95.47	23.29
14	Carbonic anhydrase 2	1I90	Thr 199(A)	OG1	y	y	-5.71	-7.72	-0.09	-0.15	64.79	2.19
15	Glucocorticoid receptor	1P93	Arg 611(A)	NH2	y	y	-5.64	-11.14	-0.15	-0.08	73.23	6.8
16	Dual specificity mitogen-activated protein kinase kinase 1	1S9J	Lys 97(A)	NZ	y	y	-3.9	-7.04	-0.23	-0.4	1.39	6.94
17	cAMP-specific 3,5-cyclic phosphodiesterase 4B	1XMU	Gln 443(A)	NE2	y	y	-5.44	-5.54	-0.02	-0.01	101.31	86.19
18	ADAM 17	1ZXC	Gly 349(A)	O	y	y	-5.85	-7.26	-0.05	-0.12	51.62	4.78
19	Mineralocorticoid receptor	2AA6	Arg 817(A)	NH2	y	y	-5.44	-6.54	-0.21	-0.08	102.53	16.16
20	Mitogen-activated protein kinase 14	2GF5	Met 109(A)	N	y	y	-5.45	-8.07	-0.07	-0.07	100.86	1.21
21	Mitogen-activated protein kinase 10	3FV8	Met 149(A)	N	y	y	5.26	-6.75	-0.06	0.37	139.84	11.33
	Anti-obesity Target											
22	Corticosteroid 11-beta-dehydrogenase isozyme 1	3BEL	Met 793(A)	N	y	y	-5.05	-2.66	-0.07	-0.04	197.11	11.22
	Anti-oxidant Targets											
23	Glutathione S-transferase A1	1GUH	Val 55(A)	N	y	y	-5.76	-8.23	-0.15	-2.65	60.22	932.43
			Thr 68(A)	N	y	y	-6.11	-8.93	-0.15	-3.41	33.45	286.46
24	Aldo-keto reductase family 1 member C2	1IHI	His 117(A)	NE2	y	y	-4.77	-10.17	-0.04	-0.26	320.69	35.31
25	Glutathione S-transferase P	2PGT	Gln 64(A)	OE1	y	y	-4.6	-5.83	-0.4	-0.2	427.64	53.01
	Anti-thrombotic Targets											
26	Prothrombin	1H8I	Gly 216(H)	N	y	y	-4.64	-1.59	-0.06	-0.02	394.6	68.57
	Cell Cycle Targets											
27	Cell division protein kinase 2	1OIQ	Leu 83(A)	O	y	y	-5.2	-6.42	-0.09	0.09	154.45	19.83
28	Cyclin-A2	2C5V	Leu 83(A)	O	y	y	-5.5	-7.03	-0.07	-0.1	93.13	7.01
	Other Important Targets for Cancer											
29	Deoxycytidine kinase	1P62	Gln 97(B)	OE1	y	y	-5.17	-7.36	-0.05	-0.31	161.6	4.03
30	Proto-oncogene tyrosine-protein kinase Src	1Y57	Met 341(A)	N	y	y	-4.97	-7.2	-0.07	-0.6	308.93	5.28
31	Proto-oncogene tyrosine-protein kinase LCK	2OG8	Met 319(A)	N		y	-5.54		-0.1		36.31	
			Asp 382(A)	N	y			-6.38		-0.81		20.98

	Nuclear Receptors										
32	Androgen receptor	1GS4	Arg 752(A)	NH2	y	y	-5.36	-9.05	-0.13	-0.36	118.62	233.78
33	Retinoic acid receptor RXR-beta	1H9U	Arg 387(A)	NH1	y	y	-5.18	-6.34	-0.17	-1.08	159.14	22.47
34	Nuclear receptor subfamily 1 group I member 2	1ILH	Ser 247(A)	OG	y	y	-4.26	-7.14	-6.38	-0.06	759.43	5.82
35	Estrogen receptor beta	1NDE	Arg 346(A)	NH2	y	y	-5.1	-0.28	-0.15	-0.21	181.42	624.52
36	Peroxisome proliferator-activated receptor gamma	1RDT	Ser 289(D)	OG	y	y	-6.07	67.79	-0.1	-0.04	35.41	48.18
37	Nuclear receptor ROR-alpha	1SOX	Arg 370(A)	NH1	y	y	-5.6	-2.26	-0.07	0.25	78.32	22.11
38	Vitamin D3 receptor	1S19	Arg 274(A)	NH1	y	y	-5.81	2.57	-0.13	0.25	-	54.68
39	Mast/stem cell growth factor receptor	1T46	Thr 670(A)	OG1	y	y	-5.96	8.98	-0.06	-0.65	42.68	-
40	Epidermal growth factor receptor	1XKK	Met 793(A)	N	y	y	183.29	175.9	0.27	-0.01	-	-
41	Basic fibroblast growth factor receptor 1	2FGI	Ala 564(A)	N	y	y	-4.89	-5.04	-0.04	0.2	260.88	201.59
42	Thyroid hormone receptor beta	2J4A	Arg 320(A)	NH1	y	y	-6.63	-13.89	-0.21	-1.8	13.71	65.74
43	Vascular endothelial growth factor receptor 2	2OH4	Glu 883(A)	OE2	y	y	-5.23	-4.23	0.03	-0.82	147.21	799.03
	Other Targets for CVD											
44	Stromelysin-1 (MMP3)	1D8F	His 711(B)	NE2	y	y	-5.57	-9.39	-0.25	-0.15	82.34	130.88
45	Estradiol 17-beta-dehydrogenase 1	1I5R	Tyr 155(A)	OH	y	y	-5.48	-6.77	-0.18	0	95.71	10.99
	Signal Transduction Modulators											
46	Peptidyl-prolyl cis-trans isomerase FKBP1A	1BL4	Tyr 82(A)	OH	y	y	-5.01	-4.24	0.07	0.07	212.33	775.28
47	Serine/threonine-protein phosphatase PP1-gamma catalytic subunit	1JK7	Tyr 272(A)	OH	y	y	-4.5	-2.77	-0.13	0.05	503.5	9.39
48	Phosphoenolpyruvate carboxykinase, cytosolic [GTP]	1M51	Asn 533(A)	ND2	y	y	-6.98	-10.51	-0.11	-0.01	7.65	19.8
49	Tyrosine-protein phosphatase non-receptor type 1	1NWL	Arg 221(A)	N	y	y	-4.38	-5.11	-0.12	-0.38	610.66	178.33
50	GTPase HRas	1P2S	Ala 18(A)	N	y	y	-4.96	-9.64	0	-2.35	232.64	86.38
51	cAMP-dependent protein kinase catalytic subunit alpha	1XH5	Val 123(A)	N	y	y	-5.53	4.46	-0.09	-0.48	88.77	-
52	Proto-oncogene tyrosine-protein kinase ABL1	2F4J	Asp 381(A)	OD1	y	y	-4.96	-10.42	0.01	-1.08	229.48	23.06

53	Angiopoietin-1 receptor	2P4I	Ala 905(A)	O	y	y	-4.39	-7.69	-0.02	0.01	608.33	2.33
54	Heat shock protein HSP 90-alpha	2VCJ	Thr 184(A)	OG1	y	y	-4.93	-10.48	-0.11	-0.61	244.65	20.68
55	Receptor tyrosine-protein kinase erbB-4	3BBT	Met 774(B)	O	y	y	-4.29	-5.36	-0.07	-0.04	718.19	117.67
	Other Important Biological Targets											
56	Serine hydroxymethyltransferase, cytosolic	1BJ4	Ser 121(A)	N	y	y	-3.84	-5.22	0.1	-2.21	1.54	149.13
57	Histo-blood group ABO system transferase	1R7U	Glu 303(A)	OE2	y	y	-4.04	-4.66	-0.05	-0.11	1.09	384.56
58	S-methyl-5-thioadenosine phosphorylase	1SD2	Met 196(A)	N	y	y	-4.93	-7.63	-0.08	-0.01	243.56	2.53
59	Beta-secretase 1	2IQG	Thr 72(A)	N	y	y	-5.28	-9.97	-0.2	-0.8	133.76	49.18

3.2 Screening Results of AutoDock

The probable 90 target molecules obtained were further screened with molecular docking using AutoDock to access the best possible targets. After docking of the target proteins, their analysis was carried out. From the analysis the binding energy, electrostatic energy and inhibition constant for ajoene and the native ligand was derived. On the basis of this information, the target proteins which showed a low binding energy are considered to be highly efficient. 59 molecules belonging to 15 classes of protein were docked successfully and their output was recorded as in the **Supplementary Table 2**.

3.3 Docking Results for the Native Ligand RIE in HMG CoA (2R4F)

According to WHO report cardiovascular diseases are the major cause of deaths worldwide. It is associated with other related diseases like atherosclerosis, hypertension, diabetes, thrombosis and myocardial infarction (MI). 3-hydroxy-3-methylglutaryl-coenzyme A reductase (HMG-CoA) is an important target in cardiovascular diseases namely with atherosclerosis [19], coronary heart disease [20], hypertriglyceridemia [21], myocardial infarction [22] and hypercholesteremia [23]. This enzyme is plays an important role in the production of cholesterol and other isoprenoids. Hence inhibitors of this enzyme help in over expression LDL (Low Density Lipoproteins) receptors in the liver, which consequently raises the catabolism of plasma LDL and lower the plasma concentration of cholesterol.

Despite the fact that AutoDock is validated in several simulation studies we in this study examined the consistency by removing the native ligand RIE from the structure and docking back to the same grid that was used for ajoene. **Fig 2** shows the PDBsum interaction of original RIE with 2R4F. In this the RIE forms 11 bonds with 2R4F with residues Lys692(A), Lys735(B), Lys691(A), Ser684(A), Asn755(B), Glu559(B), Ser565(B), Ser661(A), Arg590(A), Asp690(A).

In the **Fig 3** we can see the interaction of HMG-CoA with docked RIE and ajoene. RIE an HMG-CoA inhibitor forms four hydrogen bonds at Ser684 (2.188A^0), Lys735 (2.040A^0), Lys691 (1.87 A^0) and Asn755 (2.164 A^0). The docked interactions of ajoene with HMG-CoA showed binding at Ser684 (2.13A^0), Lys735 (1.79A^0) and Lys 691 (2.203 A^0). Thus our results reveal that ajoene can act as competitive inhibitor for HMG-CoA thus helping in reducing the complications of cardiovascular diseases. This data supports the anti-thrombotic effects of ajoene.

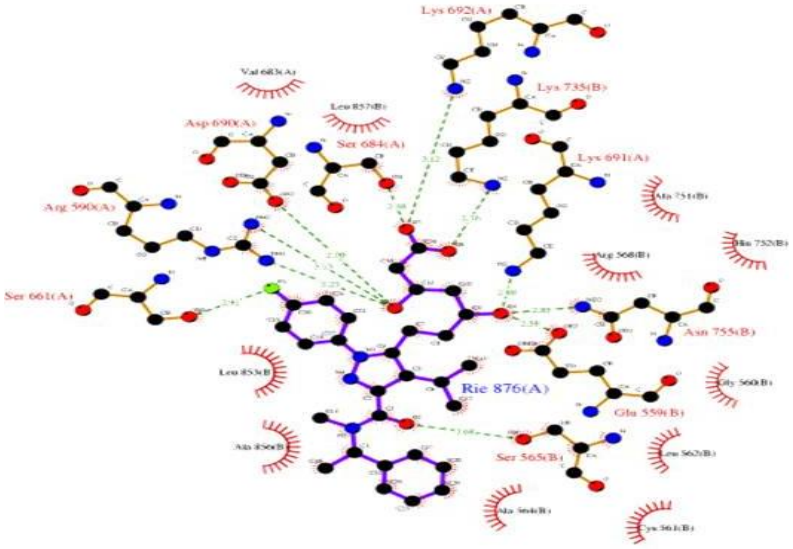

Fig 2. PDBsum LigPlot image of 2R4F vs RIE

a) **b)**

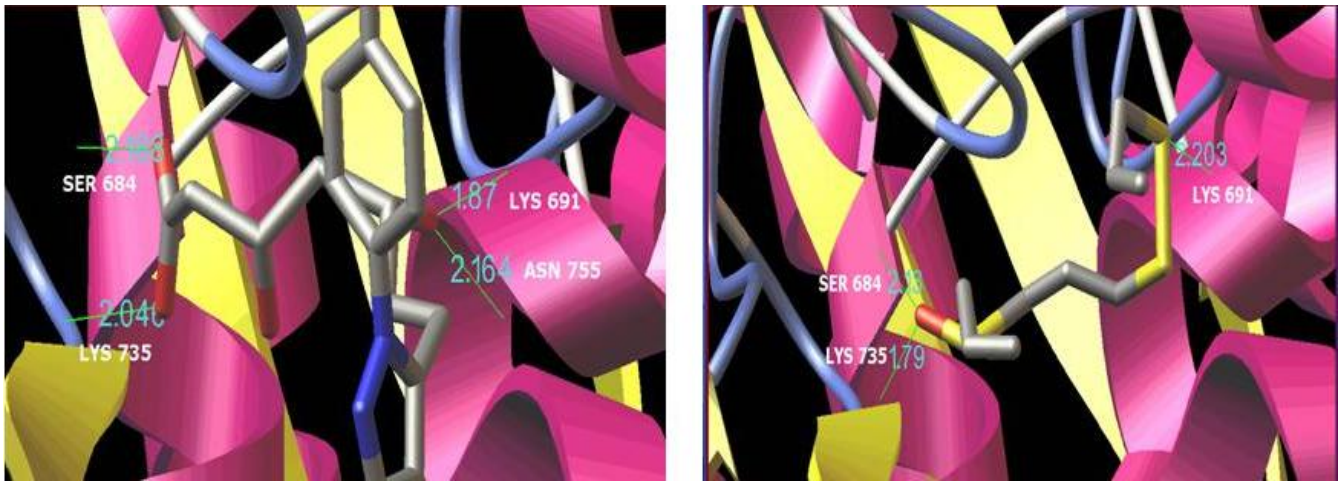

Fig 3: a) Interactions of docked ligand RIE with HMG-CoA.
b) Interactions of ajoene with HMG-CoA with 2R4F at the same grid of original PDBsum dimensions

Thus present study uses insilico screening approach using PharmMapper and PharmaGist and AutoDock to find out the potential molecular targets for ajoene. This study has revealed the molecular targets belonging to the therapeutic classes such as anti-inflammatory, anti-oxidant, anti-hypercholesteromic, anti-cancer and signal transducing modulators which confirms the therapeutic value of ajoene. This work has also identified targets that are involved in other clinical targets or drug design targets. This result verifies targets obtained from the in vivo and in vitro studies which point out ajoene having medicinal properties and can be used as a non-conventional medicine. The binding of the target molecules to ajoene signifies its therapeutic property and use in medications. The study confirms the anti-inflammatory property and also anti-cancer, anti-hypercholesteromic property of ajoene. Hence it can be used as one of the key nutraceuticals to cure people suffering from these diseases. This work may act as a platform to study and explore clinical significance of ajoene in number of diseases.

Therefore this approach can be an alternative to reveal therapeutic targets of phytochemicals of food and medicinal plants origin.

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