Available online at http://www.journalcra.com

International Journal of Current Research
Vol. 3, Issue, 11, pp.322-325, October, 2011

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

ISSN: 0975-833X

RESEARCH ARTICLE

EXTRACTION OF PHYTOCHEMICAL COMPOUNDS FROM PHYLLANTHUS EMBLICA AND DRUG DESIGNING FOR MYCOBACTERIUM TUBERCULOSIS

[1]Nancy Joy, R., [*, 2]Ramanathan, K., [1]Kalivani, R. and [1]Jeyasree, J.

[1]Department of Biotechnology, Thanthai Hans Roever College, Perambalur
[2]Department of Bioinformatics, Thanthai Hans Roever College, Perambalur

ARTICLE INFO

Article History:
Received 25th June, 2011
Received in revised form
28th July, 2011
Accepted 17th September, 2011
Published online 30th October, 2011

Key words:
Phytochemical Compounds,
Phyllanthus emblica, GCMS,
Hydrophobic Nature,
Docking.

ABSTRACT

The Phytochemical compounds from *Phyllanthus emblica* to be identified by GCMS technique. It will show the variety of phytochemical compounds. The CDCS protein which is responsible for mycobacterium tuberculosis and it was retrieved from database. The active site of the protein was calculated by PROSITE tool. The CDCS protein which has only one binding site. The lists of drugs treated for mycobacterium tuberculosis to be retrieved from databank. The hydrophobic nature of the list of drugs to be calculated by ALOGPS tool. The receptor CDCS can be docked with the ligand by Hex tool.

INTRODUCTION

The use of herbs as cures for human ailments is as old as modern man, but has now been replaced by synthetic drugs. The mixture of bioactive substances found in many traditional herbal remedies has similarities with modern combination therapies, for the treatment of tuberculosis. It is suggested that, in the development of these therapies, it might be possible to learn from the drug cocktails found in plants (Ref 1).

TUBERCULOSIS

Tuberculosis is a contagious disease caused by a bacterial infection of the lungs, which can also spread to other parts of the body, such as the brain, kidneys, and bones. Tuberculosis is caused by the bacterium *Mycobacterium tubercle*. It is contagious and spreads to others when an infected person coughs or sneezes. This shoots droplets contaminated with *Mycobacterium tubercle* bacteria into the air where they can be breathed in by others. Tuberculosis still remains one of the most deadly infectious diseases. The emergence of drug resistant strains has fuelled the quest for novel drugs and drug targets for its successful treatment. Thymidine monophosphate kinase lies at the point where the salvage and de novo synthetic pathways meet in nucleotide synthesis (Ref 2). In traditional veterinary pharmacopoeia, 18 phytogenetic species are used with 33 therapeutic indications to treat diseases including trypanosomiasis, tuberculosis, diarrheas and wounds. The interest of people of this area for medicinal plants, command a special attention to organize the actors and preserve the plant genetic resources (Ref 3).

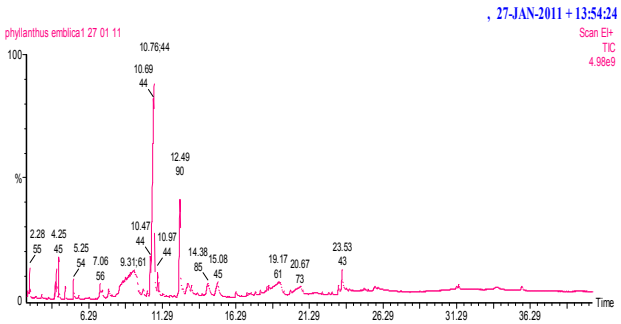
***Corresponding author:** ramanathanbio@gmail.com*

Phyllanthus emblica

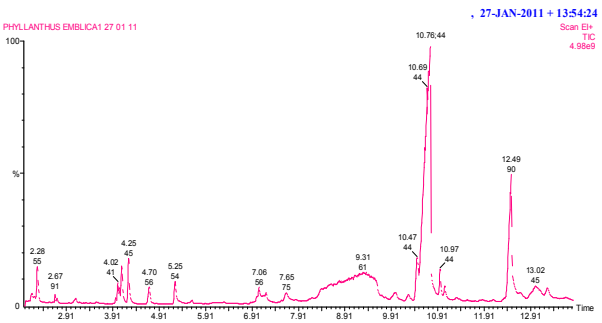
Phyllanthus emblica L apart from its food value can be used as a gastro protective agent in non steroidal anti-inflammatory drug induced gastropathy. It has been suggested that the antioxidative property of *Phyllanthus emblica L* is the key to its therapeutic effect. Hence, on the basis of in vitro antioxidative potential, the ethanolic extract of *Phyllanthus emblica L* was selected for in vivo study in induced ulcer (Ref 4). Extract of *Phyllanthus emblica* fruit and ascorbic acid were evaluated separately for protection against clastogenicity induced by lead and aluminium (Al) salts on mouse bone marrow chromosomes. Oral administration of *Phyllanthus* fruit extract (PFE) for 7 days before exposure to both metals by intraperitoneal injection increased the frequency of cell division and reduced the frequency of chromosome breaks significantly (Ref 5). In the recent investigation, aqueous extract of edible dried fruits of *Phyllanthus emblica*, a well known medicinal plant, was fed to Mus musculus for seven consecutive days prior to treatment with different doses of nickel chloride the fruit extract significantly reduced the frequency of CA/cell, the percentage of aberrant cells and the frequency of micronuclei induced by all doses of nickel in the bone marrow cells of mice (Ref 6). Correct genotype identification of medicinal plant material remains important for botanical drug industry. Limitations of chemical and morphological approaches for authentication have generated need for newer methods in quality control of botanicals. The present study was carried out to develop DNA based marker for identification of *Phyllanthus emblica* (Ref 7). The study investigated the chemistry and antioxidant properties of four commercial *E. officinalis* fruit extracts in order to determine if there are any qualitative-quantitative differences. All extracts produced positive responses in the total phenol, total flavonoid

Table 1: Phytochemical Compounds identified from *Phyllanthus emblica* by Gas Chromatography and Mass Spectroscopy

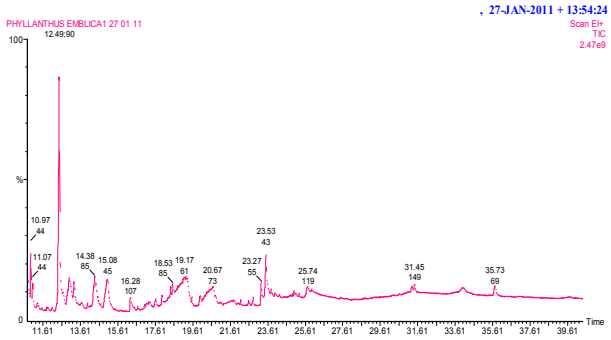
S.No.	Peak Name	Retention time	Peak area	% Peak area
1.	2-Propenoic acid Formula: $C_3H_4O_2$ MW: 72	3.11	6852926	0.3244
2.	1H-Pyrrole, 1-methyl- Formula: C_5H_7N MW: 81	3.33	450651	0.0213
4	2H-Pyran, tetrahydro-2-methyl- Formula: $C_6H_{12}O$ MW: 100	4.02	16637174	0.7874
3.	2,3-Butanediol, [R-(R*,R*)]- Formula: $C_4H_{10}O_2$ MW: 90	4.25	18128594	0.8580
4.	3,3-Dimethylbutane-2-ol Formula: $C_6H_{14}O$ MW: 102	4.70	17276210	0.8177
5.	Maleic anhydride Formula: $C_4H_2O_3$ MW: 98	5.25	23050363	1.0910
6.	3-Methyl-3-butenoic acid Formula: $C_5H_8O_2$ MW: 100	5.62	3381786	0.1601
7.	2-Butenoic acid, 3-methyl- Formula: $C_5H_8O_2$ MW: 100	5.90	106977	0.0051
8.	Butyrolactone Formula: $C_4H_6O_2$ MW: 86	6.30	1031775	0.0488
9.	2(3H)-Furanone, dihydro-5-methyl- Formula: $C_5H_8O_2$ MW: 100	7.06	20957356	0.9919
10.	Carbamic acid, phenyl ester Formula: $C_7H_7NO_2$ MW: 137	7.85	2594432	0.1228
11.	Glycerin Formula: $C_3H_8O_3$ MW: 92	9.32	483875776	22.9021
12.	2,4-Methylene-D-epirhamnitol Formula: $C_7H_{14}O_5$ MW: 178	10.01	16291174	0.7711
13.	Butanedioic acid, monomethyl ester Formula: $C_5H_8O_4$ MW: 132	10.28	8692142	0.4114
14.	Pentanal Formula: $C_5H_{10}O$ MW: 86	10.76	744969664	35.2598
15.	Pentanal Formula: $C_5H_{10}O$ MW: 86	10.97	23233304	1.0996
16.	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl- Formula: $C_6H_8O_4$ MW: 144	11.07	17098196	0.8093
17.	3,4-Dihydro-2H-pyran-2-carboxylic acid Formula: $C_6H_8O_3$ MW: 128	11.32	6310819	0.2987
18.	Benzenecarboxylic acid Formula: $C_7H_6O_2$ MW: 122	11.55	1200343	0.0568
19.	dl-Allo-cystathionine Formula: $C_7H_{14}N_2O_4S$ MW: 222	11.87	3518998	0.1666
20.	Thiophene, 2,3-dihydro- Formula: C_4H_6S MW: 86	12.11	2487164	0.1177
21.	Pentanoic acid, 2-hydroxy-3-methyl-, methyl ester Formula: $C_7H_{14}O_3$ MW: 146	12.49	209988464	9.9388
22.	1-Deoxy-d-arabitol Formula: $C_5H_{12}O_4$ MW: 136	13.02	35104804	1.6615
23.	2,5-Monomethylene-l-rhamnitol Formula: $C_7H_{14}O_5$ MW: 178	13.27	6575306	0.3112
24.	Methyl-à-d-ribofuranoside Formula: $C_6H_{12}O_5$ MW: 164	13.67	7820009	0.3701
25.	Ethanone, 1-(2-hydroxy-5-methylphenyl)- Formula: $C_9H_{10}O_2$ MW: 150	14.01	3106814	0.1470
26.	2-Deoxy-D-galactose Formula: $C_6H_{12}O_5$ MW: 164	14.38	53261028	2.5209
27.	D-Erythro-Pentose, 2-deoxy- Formula: $C_5H_{10}O_4$ MW: 134	15.03	61171636	2.8953
28.	1,4-Benzenediol, 2-methoxy- Formula: $C_7H_8O_3$ MW: 140	15.76	689160	0.0326
29.	Benzeneethanol, 4-hydroxy- Formula: $C_8H_{10}O_2$ MW: 138	16.28	16846498	0.7974
30.	Naphthalene, 1,2,4a,5,6,8a-hexahydro-4,7-dimethyl-1-(1-methylethyl)- Formula: $C_{15}H_{24}$ MW: 204(à-Amorphene)	16.68	1794056	0.0849
31.	Naphthalene, 1,2,4a,5,6,8a-hexahydro-4,7-dimethyl-1-(1-methylethyl)-, (1à,4aà,8aà)- Formula: $C_{15}H_{24}$ MW: 204	17.04	2459857	0.1164
32.	dl-Aspartic acid, N-acetyl- Formula: $C_6H_9NO_5$ MW: 175	17.27	1457134	0.0690
33.	Cadala-1(10),3,8-triene Formula: $C_{15}H_{22}$ MW: 202	17.79	144961	0.0069
34.	D-Arabinitol Formula: $C_5H_{12}O_5$ MW: 152	19.17	87863248	4.1586
35.	à-Cadinol Formula: $C_{15}H_{26}O$ MW: 222	19.57	2655690	0.1257
36.	Inositol, 1-deoxy- Formula: $C_6H_{12}O_5$ MW: 164	20.01	9348994	0.4425
37.	d-Mannose Formula: $C_6H_{12}O_6$ MW: 180	20.67	55228580	2.6140
38.	7-Hexadecenoic acid, methyl ester, (Z)- Formula: $C_{17}H_{32}O_2$ MW: 268	22.60	3372923	0.1596
39.	Hexadecanoic acid, methyl ester Formula: $C_{17}H_{34}O_2$ MW: 270(Palmitic acid, methyl ester)	22.84	4561448	0.2159
40.	Hexadecenoic acid, Z-11- Formula: $C_{16}H_{30}O_2$ MW: 254	23.27	16624145	0.7868
41.	n-Hexadecanoic acid Formula: $C_{16}H_{32}O_2$ MW: 256(Palmitic acid)	23.53	63752716	3.0174
42.	2,6-Piperazinedione, 4,4'-(1-methyl-1,2-ethanediyl)bis-, (S)- Formula: $C_{11}H_{16}N_4O_4$MW: 268(Desrazoxane)	23.80	12750357	0.6035
43.	1,4-Dioxaspiro[4.5]decane-7-methanol Formula: $C_9H_{16}O_3$ MW: 172	24.00	9737538	0.4609
44.	1-Cyclohexene-1-carboxylic acid, 4-(1,5-dimethyl-3-oxohexyl)-, methyl ester, Formula: $C_{16}H_{26}O_3$ MW: 266	24.23	3259500	0.1543
45.	15-Octadecenoic acid, methyl ester Formula: $C_{19}H_{36}O_2$ MW: 296	25.01	3822706	0.1809
46.	Octadecanoic acid, methyl ester Formula: $C_{19}H_{38}O_2$ MW: 298	25.29	2225477	0.1053
47.	Octadecanoic acid Formula: $C_{18}H_{36}O_2$ MW: 284	25.95	1962990	0.0929
48.	9,12-Octadecadienoyl chloride, (Z,Z)- Formula: $C_{18}H_{31}ClO$ MW: 298	33.97	12635526	0.5980
49.	5,7,9(11)-Androstatriene, 3-hydroxy-17-oxo- Formula: $C_{19}H_{24}O_2$ MW: 284	39.67	4437123	0.2100
50.				100.0000



Chromatogram 1



Chromatogram 2



Chromatogram 3

Fig 1: Geometrical Representation of Phytochemical compounds

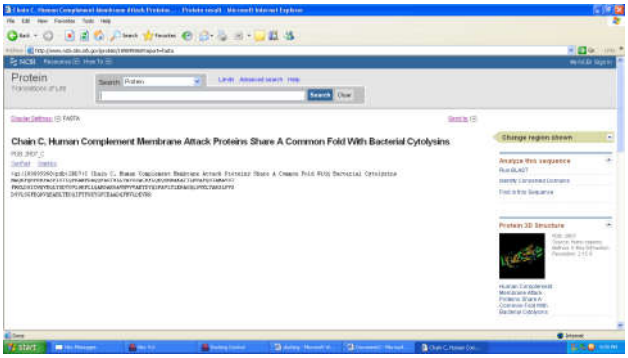


Fig. 2:Protein Sequence Retrieved from NCBI Database which isresponsible for Mycobacterium Tuberculosis

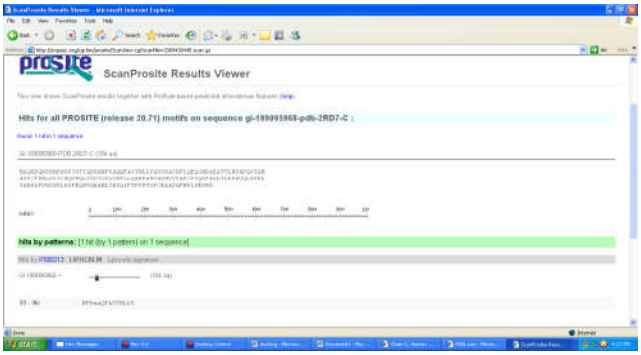


Fig 3: Binding Sites of the receptor was calculated by PROSITE tool. This protein sequence which has only one binding site

Fig 4: This shows that the Position of Binding site present in the protein sequence. The regions from 22-36 which receives the interactive

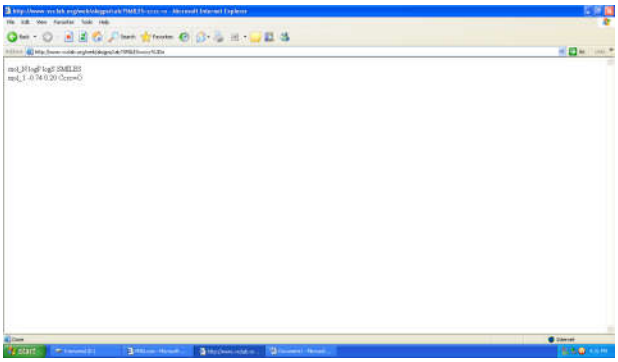


Fig 5: The Hydrophobic nature of the drug molecules can be calculated by ALOGPS tool

Table 2: The comparison of Log P and LogS Values. Among the variety of drugs Deoxy Arabitol has (1.41)highest Log P value

S.No	**Ligand**	Log P	Log S
1	Pentanal	0.42	-0.76
2	Pentnoic acid	-2.32	-0.07
3	Mannose	-2.57	0.64
4	De oxy arabitol	1.41	-0.55
5	deOxy galactose	-2.73	0.49

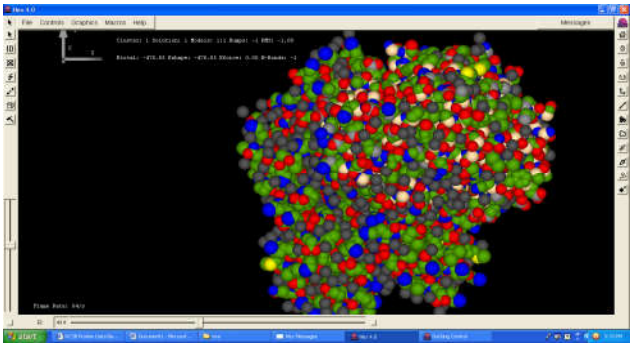


Table 5: This table shows that the comparison of Log P and Log S Values. Among the variety of drugs Deoxy Arabitol has (1.41) highest Log P value

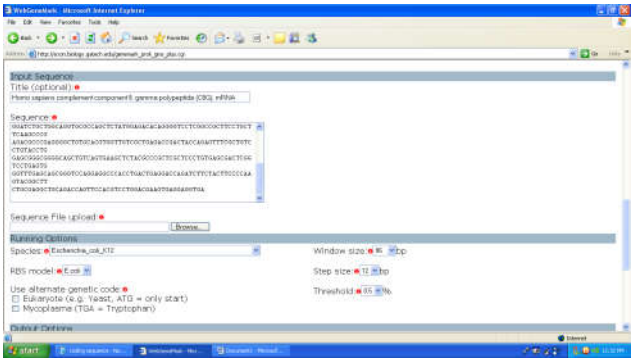


Fig 6: The Protein CDCS can be docked with the ligand Deoxy Arabitol by Hex tool

and total tannin assays. The presence of predominantly (poly) phenolic analytes, e.g. ellagic and gallic acids and corilagin, was confirmed by RP-HPLC coupled with photodiode array detection (Ref 8).

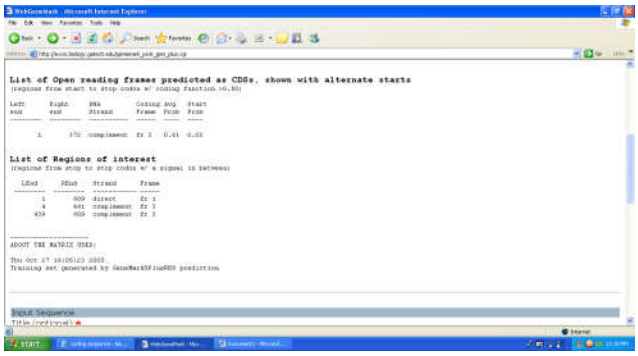


Fig 7: Gene sequence has been submitted to Gene mark tool for the identification of ORF

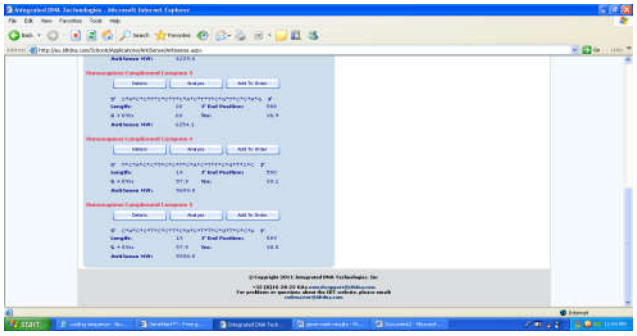


Fig 8: This shows that the regions of open reading frames present in the gene sequence. The number of frames was also calculated by Gene Mark Tool

DRUG DESIGNING AND DOCKING

A combination of protein-ligand docking and ligand-based QSAR approaches has been elaborated, aiming to speed-up the process of virtual screening. In particular, this approach utilizes docking scores generated for already processed compounds to build predictive QSAR models that in turn assess hypothetical target binding affinities for yet undocked entries. This progressive-docking procedure therefore substantially accelerates high throughput screening, especially when using high accuracy docking approaches and large-sized datasets, and has allowed us to identify several novel potent nonsteroidal SHBG ligands (Ref 9). In the current study, the applicability and scope of descriptor based QSAR models to complement virtual screening using molecular docking approach have been applied to identify potential virtual screening hits targeting DNA gyrase A from Mycobacterium tuberculosis, an effective and validated anti-mycobacterial target. (Ref 10)

METHODOLOGY

Phyllanthus emblica act as a herbal plant .It contain many compounds and it was collected from Perambalur. Then kept it at room temperature for 24 hours and then dried into powder form with the help of mixer. GC-MS is used to identify the different substances with in test sample. The samples were subjected to Gas chromatography and Mass Spectroscopy technique for the identification of phytochemical compounds. It shows the variety of phytochemicals present in *Phyllanthus emblica*. Then the list of drugs treated for Mycobacterium Tuberculosis retrieved from databank. Then we short listed the drugs based on hydrophobic activity and it is calculated by ALOGPS tool. The drug which has the hydrophobic activity was chosen for the process of docking. In our investigation, Deoxy arabitol shows the highest Log P value and it is subjected to docking. The Open Reading Frames was also calculated by Gene Mark tool. Finally, the results were compared and discussed.

CONCLUSION

The Phytochemical compounds from Phyllanthus emblica was identified by GCMS technique. It shows the variety of phytochemical compounds. Among the variety of phytochemicals deoxy arabitol shows the highest peak value. The CDCS protein which is responsible for mycobacterium tuberculosis and it was retrieved from database. The active site of the protein was calculated by PROSITE tool. The CDCS protein which has only one binding site. The lists of drugs treated for mycobacterium tuberculosis were retrieved from databank. The hydrophobic nature of the list of drugs were calculated by ALOGPS tool. The receptor CDCS can be docked with the ligand by Hex tool. From these observations, finally we concluded that deoxy arabitol has the best ligand for *Mycobacterium tuberculosis.*

REFERNCES

1. Butler A. and Keating R. 2011. Old herbal remedies and modern combination therapy. *Scott Med J.,* 56(3):170-3.
2. Chandra K, Dutta D, Das AK, Basak A. 2010. Design, synthesis and inhibiton of activity of novel cyclic peptides against protein tyrosine phosphatase A from Mycobacterium tuberculosis. *Bioorg Med Chem.,* 1; 18 (23):8365- 73.
3. Ky JM, Zerbo P, Gnoula C, Simpore J, Nikiema JB, Millogo-Rasolodimby J. 2009. Medicinal plants used in traditional medicine in the centre east region of Burkina Faso. *Pak J Biol Sci.,* 1; 12(19):1287-98.
4. Chatterjee A, Chattopadhyay S, Bandyopadhyay, 2011. Biphasic effect of Phyllanthus emblica L extract on induced ulcer. An Antioxidative Trial weaved with Immunomodulatory Effect. *Evid Based Complement Alternat Med.,* 146808.
5. Dhir H, Agrawal K, Sharma A, G.Taludker. Modification of clastogenicity of lead and aluminium in mouse bone marrow cells by dietary ingestion of phyllanthus emblica fruit extract. Mutat Res. 241(3):305 – 12. (1990)
6. Dhir H, Agrawal K,Sharma A, Taludker G. 1991. Modifying role of *Phyllanthus emblica* and ascorbic acid against nickel clastogenicity in mice. *Cancer Lett.,* 26; 59(1):9-18.
7. Dyyaneshwar W, Preeti C, Kalpana J, Bhushan P. Development and application of RAPD-SCAR marker for identification of *Phyllanthus emblica. Biol Pharm Bull.,* 29 (1):2313-6.
8. Poltanov EA, Shikov AN, Dorman HJ, Pozharitskaya ON, Markarov VG, Tikhonov VP,Hiltumen R. Chemical and antioxidant evaluation of Indian gooseberry supplements. *Phytoether Res.,* 23(9):1309-15.
9. Cherkasov A, Ban F, Li Y, Fallahi M, Hammond GL. 2006. Progressive docking: a hybrid QSAR docking approach for accelerating in silico high throughout screening. *Journal of Med Chem.,* 14; 49 (25):7466-78.
10. Singh V, Mani I, Chaudhary DK, Somvanshi P. 2011. The Beta Ketoacyl ACP Synthase from Mycobacterium tuberculosis as potential drug targets. *Curr Med Chem.,* 18(9):1318-24.