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Research article

An *in-silico* approach on essential oil molecules as apoptosis inducer in cancer chemotherapy

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Abstract

Essential oils (EOs) are very engrossing natural plant products and apart from this they possess various biological properties. It has been reported that these essential oil molecules are able to inhibit tumor cell proliferation and induce tumor cell death by inhibiting multiple cancer-specific targets including the suppression of anti-apoptotic pathways i.e., BCL-2, BCL-XL, MCL-1, and NFκb. This study was conducted with the objective of exploring the anticancer activity of herbs and spices, with special reference to its potential to inhibit anti-apoptotic pathways by studying their interaction pattern with the selective inhibitors of the particular receptors. Hence a comparative *in-silico* study was done in which the essential oil molecules were docked with specific anti-apoptotic receptors with respect to the particular known inhibitor of that receptor therefore the binding affinity of the essential oil molecule with that of the receptor site was analyzed. It has been observed that the phytochemicals like capsaicin have an impressive binding affinity for NFκb receptor, BCL-2 as compared to its standard inhibitors, which shows that the phytochemical has stronger binding affinity for receptor. These docking results hereby apparently tells us that the binding affinity of the essential oil molecules are either comparable or more than that of the specific inhibitors of the receptors hence in future drug molecules can be synthesized keeping in view the strong binding affinity of these molecules with the receptors.

Keywords: Essential oils, Anti-apoptosis, *in-silico*, Phytochemicals. Anticancer activity

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1. Introduction

Plants play an important role in discovery of therapeutics. Since ages plants have been served as vital source for development of important drug molecules. They have always been a rich resource of lead compounds e.g. morphine, cocaine, digitalis, quinine etc. It is estimated that more than 5,000 individual photochemical identified in fruits, vegetables, grains, and other plants and are mainly classified as phenolics, carotenoids, vitamins, alkaloids, nitrogen-containing compounds, organ sulfur compounds and essential oils [1]. Among the great structural diversity of photochemical, essential oil components have attracted considerable interest and the most attention for their wide variety of bioactivities [2]. In addition, essential oils abundant in flowers, leaves, fruits, and bark are reported to play an important role as chemo-preventive agents; for example, the volatile components of *Pistacialentiscus* L. (Chios Mastic Gum) have been linked with inhibition of colon, prostate and lung cancer *in-vitro* [3]. Many volatile constituents have been reported to possess potent antioxidant activity and to have anticancer or anti-carcinogenic/anti mutagenic /anti proliferation [3-6] effects. A comprehensive study of chemical constituents of selected medicinal plants, have led to the identification of potential anticancer molecules. These essential oils molecules of natural origin may lead to higher safety and efficiency in drug development. It has been reported that these molecules are able to inhibit tumor cell proliferation and induce tumor cell death by inhibiting multiple cancer-specific targets, including activation of apoptosis triggered by inhibition NF-κB pathways consequently leading to suppression of anti-apoptotic BCL-2 proteins [7].
Apoptosis represents a physiological process cell death

involved in the regulation of tissue homeostasis [8]. During the progression of cancer, there is a malfunction of apoptosis as there are the up regulations of anti-apoptotic proteins and/or down regulation of pro-apoptotic signaling pathways, which eventually leads to poor response to conventional chemotherapy. The BCL-2 (B-cell lymphoma/leukemia-2) family including pro and anti-apoptotic proteins are key regulators of apoptosis. This family compromises of at least 26 proteins divided into three groups, or subfamilies, on the basis of their function and the composition of their BCL-2 homology (BH) domains [9, 10].

There are six anti apoptotic members of the BCL-2 family in humans (BCL-2, BCL-xl, BCL-B, BCL-W, Bfl-1, and MCL-1) and there is a hydrophobic cleft in these proteins that share homology in BH3-only proteins and to the pro-apoptotic BCL-2 family members Bad, Bak, and Bax to inhibit apoptosis. In unbounded state, the pro-apoptotic BCL-2 members are recruited to the Outer Mitochondrial Membrane (OMM) where they oligomerize releasing pro-apoptotic effectors such as cytochrome-C. The released cytochrome-C binds to APAF-1 and pro-caspase 9 to from the apoptosome, which generates mature caspase 9 and begins a proteolytic cascade, ultimately resulting in cell death [11]. In order to suppress the expression of BCL-2 anti-apoptotic proteins the inhibitors of the BCL-2 class proteins are designed and synthesized.

The NF-κB pathway is responsible for expression of genes required in control of immune and inflammatory responses [12-15] apart from that NF kappa b also is a key mediator of genes involved in the control of the cellular proliferation and apoptosis [16]. Anti-apoptotic genes that are directly activated by NF-κB include the cellular inhibitors of apoptosis (c-IAP1, c-IAP2, and IXAP), the TNF receptor–associated factors (TRAF1 and TRAF2), the BCL-2 homologue A1/Bfl-1, and IEX-IL [17, 18]. One of the best-studied pathways that activate apoptosis is induced following treatment of cells with TNF-α. TNF-α treatment increases the expression of TRAF1, TRAF2, c-IAP1, and c-IAP2 [16]. The over expression of these proteins can protect RelA-deficient cells, which are highly sensitive to TNF-α–induced apoptosis, from cell death. These anti-apoptotic proteins block the activation of caspase-8, an initiator protease, involved at an early step in stimulating the apoptotic pathway [16]. Members of the BCL-2 family may be anti apoptotic, as is the case with BCL-2, BCL-xL, and A1/Bfl-1, or pro-apoptotic, as with Bad, Bax, and BCL-xS. NF-κB directly induces expression of A1/Bf1-1 by binding to specific sites in its promoter [18]. The constitutive expression of A1/Bfl-1 inhibits antigen receptor–induced apoptosis in B-lymphocytes derived from c-Rel–deficient mice, suggesting that NF-κB activation of this protein plays an important role in the B-lymphocyte survival following lymphocyte activation [18]. The chemotherapeutic agent etoposide also increases NF-κB levels and thereby induces A1/Bfl-1, which prevents cytochrome *c* release from mitochondria and activation of caspase-3 [19]. By increasing the expression of anti apoptotic cellular proteins, NF-κB activation can thus reduce apoptosis in response to treatment with different chemotherapeutic agents.

Essential oil molecules act in the sequence of IKK beta, TRAF6 recruitment that characterizes TNF-induced NF-kappa beta (B) activation. From the comparative molecular modeling studies of these essential oil molecules and the standard known receptors it has been observed that these molecules have a high affinity for the proteins involved in the signaling pathway of NF kappa beta. NF-kappa B also regulates the expression of the anti-apoptotic proteins including BCL-2, BCL-xL and MCL-1 suppression of which can lead to apoptosis in cancer cell as it has been known that there is an over-expression of these proteins and NF kappa B in cancer cells. This study was conducted with the objective of exploring the anticancer activity of herbs and spices, with special reference to its potential to inhibit anti-apoptotic pathways.

In the present study we have docked 65 essential oil molecules on receptors participating in the process of apoptosis including the BCL-2, MCL-1 and BCL-XL anti apoptotic receptors and the NF kappa B receptor. These 65 essential oil molecules were selected because these molecules have shown significant *in-vitro* activities against different cancer cell lines. Figure 1 show depicts the structures of essential oil molecules docked with different receptors.

2. Materials and methods

Protein preparation

The starting structures for virtual study experiments of BCL-2, BCL-Xl, MCL-1 complexes and NF kappa B, ikk beta, Traf 6 and were retrieved from the RCSB with the protein data bank (codes 4AQ3, 2YXJ, 4G35, 4DN5, 3BRV, 1LB5). All bound ligands (small molecules and BH3 peptides), waters beyond 5Å and ions, molecules and hetero-atoms were removed from the complexes. Any missing disulphide bonds were added. The H-bonds were optimized using protassingn at pH 7.0. Restrained minimization was done by impref with the convergences of the heavy atoms to root mean square deviation (RMSD) of 30 Å using OPLS-2005 force field.

Ligand preparation

The ligands used were sketched by using maestro and saved in structure-data file (SDF) format. The ligands and the inhibitor were prepared by using the Ligprep application of the maestro 9.3; force field used was OPLS-2005. The inhibitors of BCL-2, BCL-xl, MCL-1 and NF kappa B were ABT 263, ABT 737, Obatoclax, Parthenolide were retrieved from Pubchem (CID 24978538, CID 11228183, CID 16681698, CID 5353864 respectively).

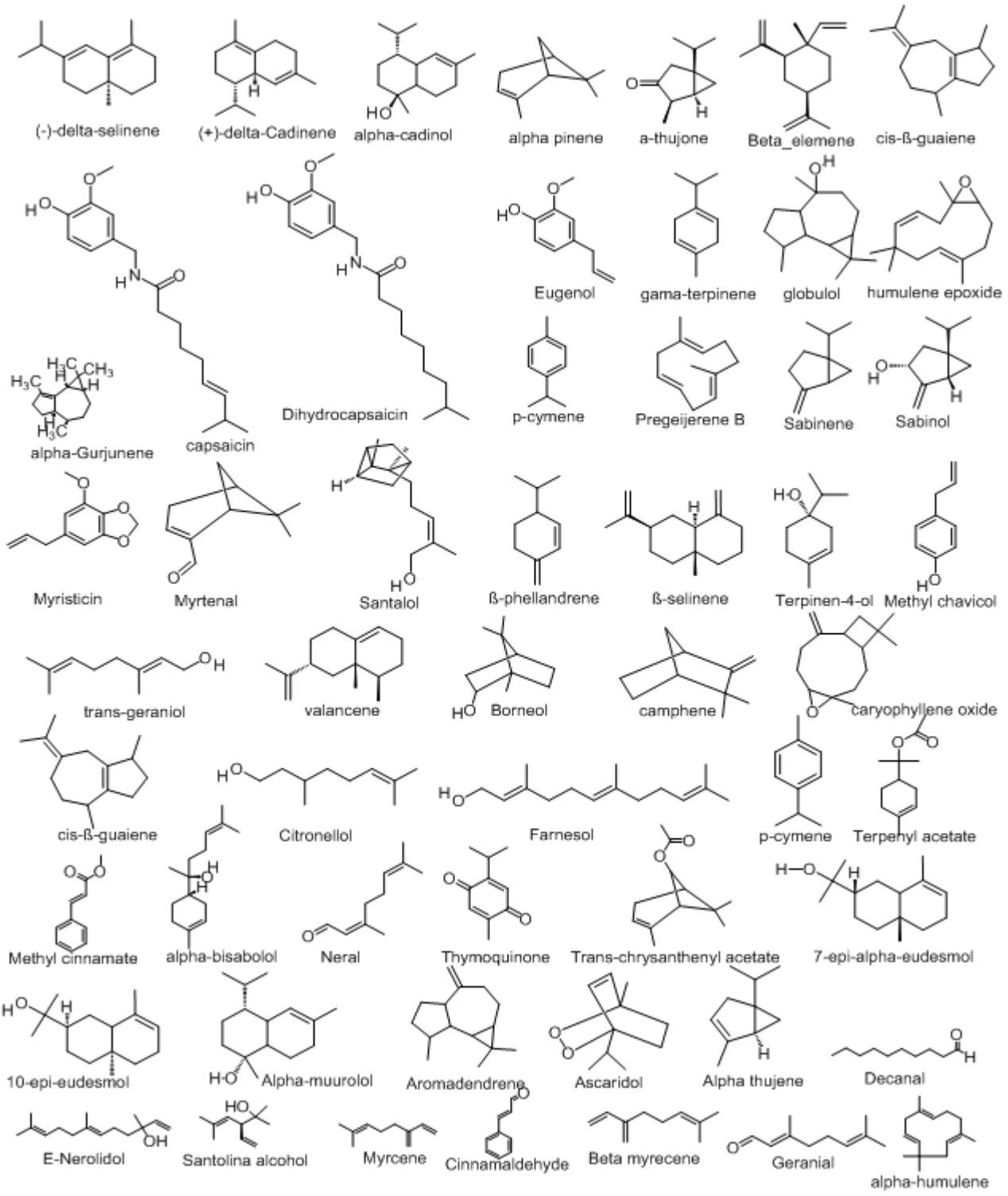

Figure 1: Structures of the essential oil molecules docked

Receptor grid generation

The active site was identified using Receptor grid generation application of Glide (1) in which Van der waals scaling was reduced to 1.0 to soften the potential for non-polar parts of the receptor with partial atomic charge cut off of 0.25. It identifies and measures pockets and pocket mouth openings, as well as cavities. The length of the ligands to be docked was increased to 36 Å.

Docking

Essential oil molecules and the inhibitors docking was

done using XP (extra precision), XP descriptors were written. Ligand was taken as flexible. Sample nitrogen inversions and sample ring conformation was taken into account. Bias sampling of torsions was one only for amides and non-polar conformations were penalized. Epik penalties were added to the docking score. Vander waalsscalling was adjusted to 0.8 and partial charge cutoff to 0.15 to soften the non-polar parts of the ligand. 10000 poses per docking run were allowed to run and 1 pose per ligand was allowed to be written. In the post docking minimization number of poses per ligand to be included was taken to be 10. The threshold energy below which the pose to be rejected was 0.5 kcal/mol. After docking, the individual binding poses of each ligands were observed and their interactions with the protein were studied. The best and most energetically favorable conformation of each ligand was selected. For the validation of the docking procedure the RMSD value was calculated after re-docking ABT-737, which was the inhibitor for BCL-XL (PDB id 2YXJ), RMSD was found to be 0.4756 Å.

3. Discussion

From the results of molecular modeling we have observe the interaction of essential oil molecules with the receptors. It has been reported that there is an over-expression of NFκB and BCL2 anti-apoptotic proteins in cancer. Many *in-vitro* studies have suggested that the essential oil molecules possess the ability to suppress these proteins but till date no interaction profile regarding these molecules have been reported so in order to clearly determine how these molecules bind to apoptotic signaling pathways including NFκB receptors and BCL2 anti-apoptotic receptors we had carried out this comprehensive study. Through this study confirmed that these molecules have a multi-target domain because they not only bind to the NFκB but also have high affinity for the receptor proteins involved in the signaling pathway including Ikkβ, traf-6. These photochemical have shown high affinity for the BCL-2 anti-apoptotic proteins as well, including the BCL-2, BCL-XL and MCL-1. Interaction profile depicts that these photochemical possess the same binding site as that of the selective inhibitors. Hence, it can be concluded that in future these molecule can be considered as potent inhibitor of anti-apoptotic proteins. Phytochemicals including Capsaicin, Dihydrocapsaicin, (±)Δ-Cadinene revealed strong binding affinity with BCL-2 anti-apoptotic protein as compared to the selective inhibitor of the BCL-2 protein ABT-263 (Figure 2). In year 2012 that capsaicin induces apoptosis in FaDu cells and this response is associated with mitochondrial pathways (BCL-2suppresion) [20]. As capsaicin, dihydrocapsaicin and (±)Δ-Cadinene have a glide dock score of -5.54kcal/mol, -4.98kcal/mol, -4.76kcal/mol respectively and that of selective inhibitor ABT 263 is -4.67kcal/mol, indicating that capsaicin has highest binding affinity for the receptor site followed by dihyrocapsaicin and (±)delta-Cadinenethan the selective inhibitor ABT263. Capsaicin binds with the receptor site via hydrogen bond formation with the side chain of the capsaicin molecule and TYR 67 residue it also forms hydrophobic interaction with the amino acid residue of LEU 96, VAL 92 PHE 109, PHE 71, PHE 112, MET 74 and ALA 108 and electrostatic interaction with ASP 62 amino acid residue (Figure 2b). The interaction reveals that the electrostatic forces and hydrophobic bonding are the forces which are responsible for the binding affinity of capsaicin with the receptor site. The results of the docking of molecules with BCL-2 receptor are depicted in Table 1.

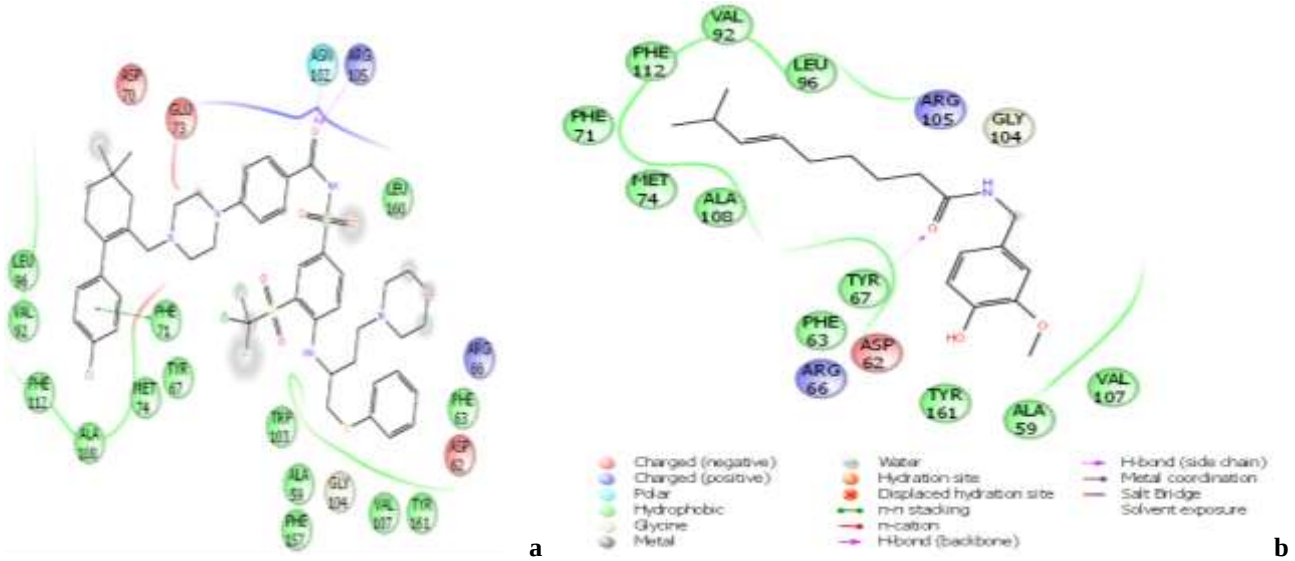

Figure 2: Interaction profile and docked poses of ABT-263 (a) and Capsaicin with BCL-2 receptor (b).

Table 1: Docking scores of essential oil molecule with BCL-2 receptor

S. No.	LIGANDS	Dock Score	Lipophilic Evdw	H Bond	Electro
1.	Capsaicin	-5.54	-3.93	-1.18	-0.18
2.	Dihydrocapsaicin	-4.98	-3.87	-1.18	-0.15
3.	(+)-delta-Cadinene	-4.76	-3.84	0	-0.01
4.	ABT-263(inhibitor)	-4.67	-5.76	-1.05	-0.57
5.	Sabinol	-4.61	-3.18	0	0.01
6.	Eugenol	-4.58	-3.03	-0.96	-0.28
7.	α-bisabolol oxide B	-4.58	-3.4	0	-0.33
8.	p-cymene	-4.46	-3.26	0	-0.04
9.	ß-phellandrene	-4.45	-3.28	0	0
10.	Myrtenal	-4.33	-2.71	0	-0.03
11.	Terpinen-4-ol	-4.21	-3.03	0	-0.05
12.	α-cadinol	-4.11	-3.08	0	-0.02
13.	(-)-delta-selinene	-4.08	-3.31	0	-0.02
14.	humulene epoxide	-4.03	-3.01	0	0.03
15.	Santalol	-3.99	-3.12	-0.17	-0.09
16.	α-pinene	-3.97	-2.83	0	-0.01
17.	trans-geraniol	-3.94	-3.3	-0.7	-0.21
18.	globulol	-3.93	-2.89	0	-0.03
19.	β –elemene	-3.92	-3.58	0	-0.03
20.	Sabinene	-3.89	-2.93	0	-0.01
21.	γ-terpinene	-3.8	-2.97	0	-0.04
22.	cis-ß-guaiene	-3.79	-3.01	0	-0.02
23.	Valancene	-3.77	-3.32	0	-0.02
24.	α - muurolol	-3.75	-2.95	0	-0.06
25.	Pregeijerene B	-3.73	-3	0	0.03
26.	α-thujone	-3.71	-2.46	0	0.01
27.	ß-selinene	-3.66	-2.72	0	-0.01
28.	Myristicin	-3.64	-2.76	0	-0.08
29.	10_epi_eudesmol	-3.64	-2.94	-0.35	-0.21
30.	pregeijerene	-3.64	-2.96	0	0.03
31.	Aromadendrene	-3.61	-3.11	0	0
32.	terpenyl acetate	-3.59	-3.28	0	0.01
33.	α-gurjunene	-3.57	-2.99	0	0
34.	Citronellol	-3.56	-3.07	-0.7	-0.2
35.	ascaridole	-3.56	-3.05	0	-0.01
36.	Thujones	-3.56	-2.42	0	0.01
37.	α-bisabolol	-3.53	-3.17	0	-0.02
38.	trans-chrysanthenyl acetate	-3.52	-2.83	0	0
39.	7-epi-a-eudesmol	-3.51	-2.65	-0.28	-0.1
40.	α-thujene	-3.51	-2.66	0	0
41.	methyl chavicol	-3.5	-3.18	0	-0.04
42.	methyl cinnamate	-3.46	-2.32	-0.7	-0.14
43.	Camphene	-3.35	-2.47	0	-0.01
44.	α-humulene	-3.33	-2.66	0	0
45.	neral	-3.31	-3.03	0	0.02
46.	Thymoquinone	-3.25	-1.99	0	0.03
47.	caryophyllene oxide	-3.13	-2.8	0	0.02
48.	Farnesol	-3.08	-3.14	-0.7	-0.21
49.	Borneol	-2.98	-2.65	0	-0.02
50.	Santolina alcohol	-2.88	-2.97	0	0
51.	Myrcene	-2.86	-3.09	0	0
52.	Geranial	-2.76	-2.84	0	0.02
53.	(E)-cinnamaldehyde	-2.75	-2.61	0	-0.01
54.	β-myrcene	-2.73	-3.05	0	0
55.	E)-Nerolidol	-2.25	-2.98	-0.62	-0.1
56.	Decanal	-0.86	-1.9	-1.05	-0.33

Santalol, dihydrocapsaicin, α-bisabolol oxide B and Capsaicin have showed comparable binding affinity for BCL-XL receptor but less than that of the selective inhibitor of BCL-XLABT 737 (Figure 3). The santalol binds with the BCL-XL receptor via hydrogen binding with water molecule present in the receptor site while it shows hydrophobic interaction with ALA 142, ALA 149, PHE 97, TYR 101, PHE 105, LEU 108, PHE 146, LEU 130 and VAL 126 amino acid residue present and it also shows electrostatic forces with GLU 129 amino acid residue on the receptor site of BCL-XL (Figure 3b). The anticancer effects of α-santalol, a major component of sandalwood oil, have been reported against the development of certain cancers such as skin cancer both *in-vitro* and *in-vivo*. Bommareddy et al., revealed in his study on the apoptotic effects of α-santalol in inhibiting the growth of human prostate cells (21). The results of the docking of molecules with BCL-XL receptors are reported in Table 2.

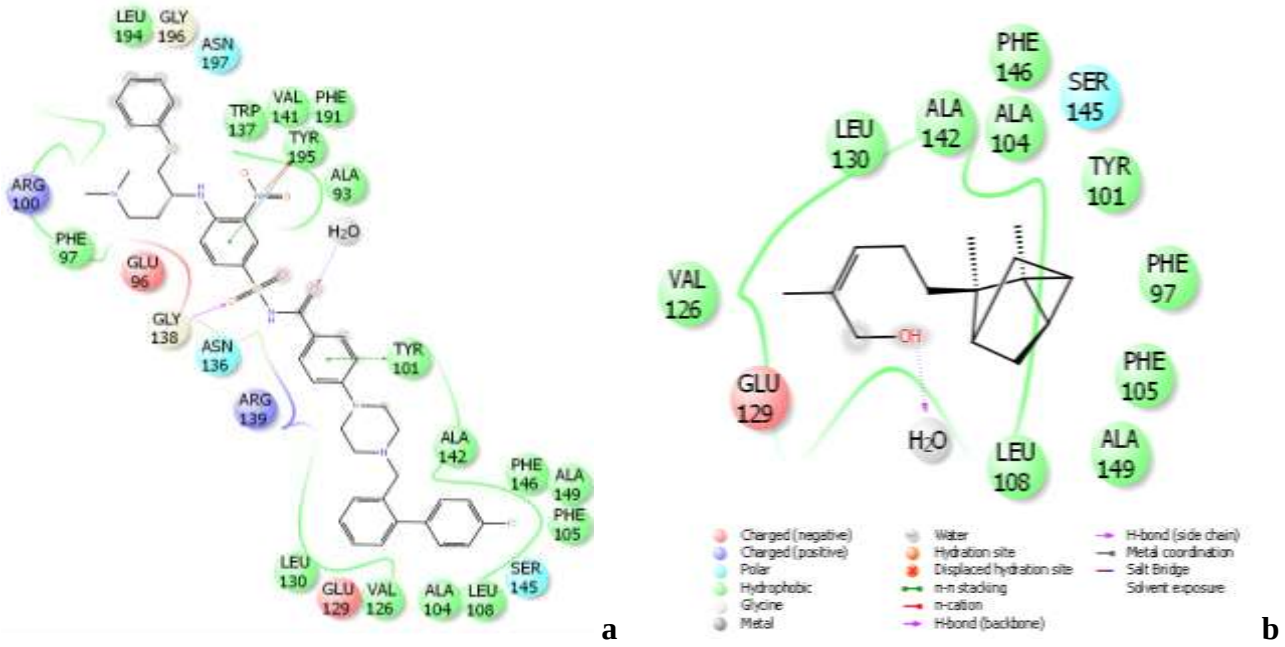

Figure 3: Interaction profile and docked poses of ADT-737 (a) and Santalol with BCL-XL receptor (b).

Table 2: Docking scores of essential oil molecule with BCL-XL receptor

S. No.	Ligand	Dock Score	Lipophilic Evdw	H Bond	Electro
1.	ADT_737(Inhibitor)	-9.47	-7.25	-0.7	-0.41
2.	Santalol	-5.9	-3.26	-0.7	-0.29
3.	Dihydrocapsaicin	-5.75	-4.13	-1.3	-0.32
4.	α-bisabolol oxide B	-5.72	-3.73	-0.48	-0.05
5.	Capsaicin	-5.41	-4.41	0	-0.18
6.	γ-terpinene	-5.25	-3.36	0	-0.04
7.	Eugenol	-5.25	-3.35	-0.81	-0.21
8.	p-cymene	-5.19	-3.26	0	-0.06
9.	Sabinol	-5.09	-2.59	-0.66	-0.22
10.	Thymoquinone	-5.03	-2.9	0	0
11.	Thujones	-5.02	-2.78	0	-0.01
12.	Terpinen-4-ol	-4.86	-2.39	-0.68	-0.19
13.	ß-phellandrene	-4.8	-3.06	0	0.01
14.	α-bisabolol	-4.75	-3.53	0	-0.05
15.	Myristicin	-4.59	-3.23	0	0.03
16.	Farnesol	-4.46	-3.59	-1.29	-0.36

S. No.	Ligand	Dock Score	Lipophilic Evdw	H Bond	Electro
17.	methyl cinnamate	-4.43	-2.84	0	-0.03
18.	Ascaridole	-4.31	-3.28	0	-0.01
19.	α-thujone	-4.22	-2.36	0	0.06
20.	α-thujene	-4.08	-2.84	0	0.01
21.	methyl chavicol	-3.96	-2.95	0	-0.05
22.	10_epi_eudesmol	-3.88	-2.63	-0.7	-0.3
23.	(+)-delta-Cadinene	-3.85	-3.33	0	0.04
24.	(E)-cinnamaldehyde	-3.78	-2.73	0	-0.04
25.	E)-Nerolidol	-3.71	-3.89	-0.44	-0.08
26.	(-)-delta-selinene	-3.69	-2.99	0	0.03
27.	Aromadendrene	-3.67	-3	0	0
28.	7-epi-a-eudesmol	-3.67	-2.46	-0.7	-0.37
29.	Sabinene	-3.63	-2.81	0	0
30.	α-cadinol	-3.62	-2.88	0	-0.05
31.	Valancene	-3.59	-3.13	0	0.01
32.	α-humulene	-3.53	-3.14	0	-0.01
33.	trans-geraniol	-3.53	-2.88	-0.61	-0.15
34.	humulene epoxide	-3.52	-3.15	0	-0.02
35.	α-gurjunene	-3.49	-3.19	0	0.03
36.	Globulol	-3.45	-3.17	0	0
37.	ß-selinene	-3.45	-2.81	0	0.03
38.	trans-chrysanthenyl acetate	-3.44	-2.29	-0.7	-0.14
39.	Pregeijerene B	-3.44	-3.13	0	0
40.	terpenyl acetate	-3.42	-2.54	-0.7	-0.19
41.	Pregeijerene	-3.39	-2.89	0	0
42.	cis-ß-guaiene	-3.38	-2.8	0	0
43.	β elemene	-3.19	-3.02	0	-0.02
44.	Citronellol	-3.18	-2.04	-1.33	-0.44
45.	α –pinene	-3.18	-2.5	0	0
46.	Geranial	-3.17	-2.64	-0.7	-0.2
47.	α- muurolol	-3.15	-2.7	0	-0.01
48.	Myrtenal	-3.06	-1.67	-0.7	-0.19
49.	caryophyllene oxide	-3.04	-2.79	0	0.01
50.	Santolina alcohol	-2.96	-2.38	-0.7	-0.18
51.	neral	-2.93	-2.46	-0.69	-0.16
52.	Myrcene	-2.69	-3.31	0	0.02
53.	Camphene	-2.67	-2.48	0	-0.01
54.	Borneol	-2.64	-2.27	0	-0.06
55.	beta-myrcene	-2.46	-2.96	0	-0.01
56.	Decanal	-1.6	-2.7	-0.65	-0.17

Whereas the photochemical Capsaicin and Dihydrocapsaicin had low affinity for MCL-1 receptor (Figure 4) than the selective inhibitor obatoclax with a little difference in the dock score. The results of the docking of molecules with the MCL-1 receptor are depicted in Table 3.

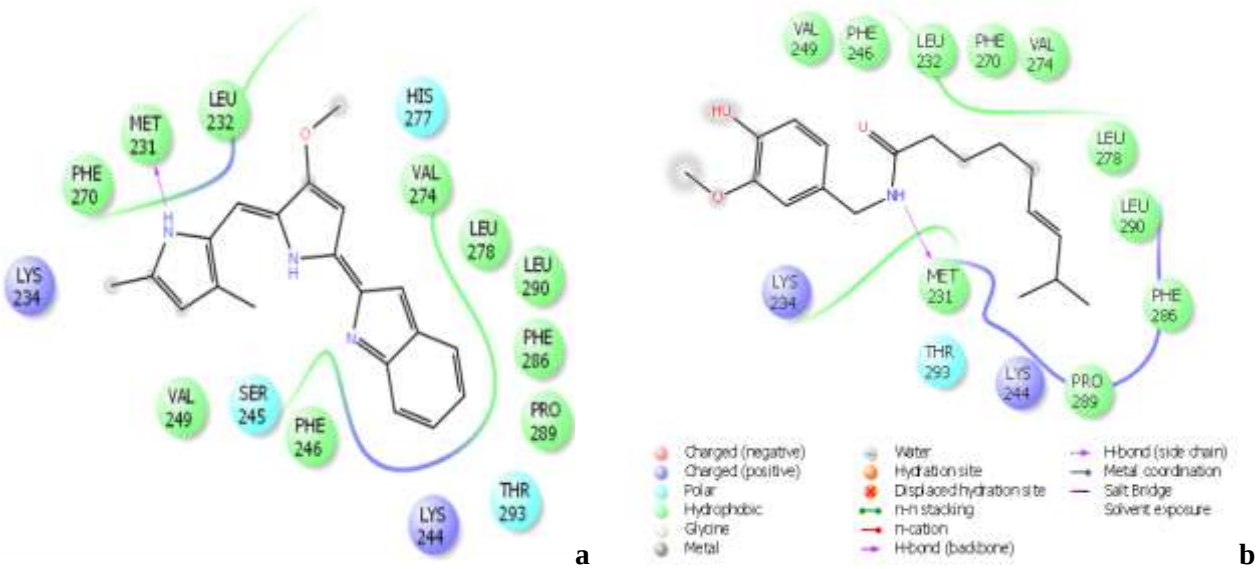

Figure 4: Interaction profile and docked poses of Obatoclax (a) and Capsaicin with MCL-1 receptor (b).

Table 3: Docking scores of essential oil molecule with MCL-1 receptor

S. No.	Ligands	Dock Score	Lipophilic Evdw	H Bond	Electro
1.	Obatoclax(Inhibitor)	-6.77	-3.11	-0.7	-0.22
2.	Capsaicin	-5.56	-4.01	-1.18	-0.29
3.	dihydrocapsaicin	-5.15	-3.87	-1.03	-0.18
4.	α-bisabolol oxide B	-4.69	-2.74	-0.96	-0.38
5.	Santalol	-4.37	-2.92	-0.69	-0.19
6.	terpenyl acetate	-4.21	-1.76	-1.42	-0.36
7.	trans-chrysanthenyl acetate	-4.08	-2.2	-0.83	-0.24
8.	10_epi_eudesmol	-4.02	-2.46	-0.58	-0.07
9.	7-epi-a-eudesmol	-3.9	-2.14	-0.98	-0.18
10.	γ-terpinene	-3.83	-2.92	0	0
11.	Sabinol	-3.82	-2.11	-0.7	-0.17
12.	p-cymene	-3.75	-2.3	0	0.01
13.	Myrtenal	-3.71	-2.34	0	-0.01
14.	Farnesol	-3.71	-3.75	-0.7	-0.23
15.	methyl cinnamate	-3.71	-1.81	-0.95	-0.24
16.	pregeijerene	-3.68	-2.64	0	0.02
17.	E)-Nerolidol	-3.67	-3.74	-0.7	-0.18
18.	ß-selinene	-3.67	-2.84	0	0.01
19.	Thujones	-3.61	-2.04	0	-0.09
20.	cis-ß-guaiene	-3.61	-2.64	0	0.02
21.	a-bisabolol	-3.49	-2.39	-0.7	-0.24
22.	ß-phellandrene	-3.45	-2.14	0	0.03
23.	thymoquinone	-3.44	-2.06	0	-0.05
24.	α- pinene	-3.44	-2.28	0	0.03
25.	β-elemene	-3.39	-3.28	0	0.04
26.	Eugenol	-3.34	-2.2	-0.96	-0.16
27.	trans-geraniol	-3.28	-2.57	-0.7	-0.2
28.	Valancene	-3.28	-2.49	0	0.02
29.	α-thujene	-3.25	-2.51	0	0.01
30.	α-cadinol	-3.21	-1.66	-0.43	-0.22
31.	Borneol	-3.21	-2.07	0	-0.01
32.	α-thujone	-3.2	-1.88	0	-0.07
33.	Santolina alcohol	-3.19	-2.26	-0.7	-0.26
34.	globulol	-3.18	-2.2	0	-0.08

S. No.	Ligands	Dock Score	Lipophilic Evdw	H Bond	Electro
35.	humulene epoxide	-3.17	-1.52	-0.7	-0.17
36.	Terpinen-4-ol	-3.14	-2.12	0	0
37.	Sabinene	-3.12	-2.22	0	0.02
38.	(E)-cinnamaldehyde	-3.12	-1.69	-0.93	-0.23
39.	α - muurolol	-3.11	-2.11	-0.42	-0.16
40.	caryophyllene oxide	-3.08	-2.22	0	0.05
41.	(+)-delta-Cadinene	-3.07	-2.34	0	0.01
42.	Pregeijerene B	-3.04	-2.16	0	0.03
43.	ascaridole	-3.02	-2.4	0	0.06
44.	aromadendrene	-3	-2.17	0	0.03
45.	methyl chavicol	-2.95	-2.55	0	0
46.	Myristicin	-2.92	-2.18	0	-0.02
47.	geranial	-2.81	-1.97	-0.98	-0.23
48.	citronellol	-2.62	-1.99	-0.67	-0.34
49.	α-gurjunene	-2.49	-1.7	0	0.03
50.	β-myrcene	-2.44	-2.87	0	-0.02
51.	neral	-2.41	-1.89	-0.59	-0.25
52.	Myrcene	-2.41	-2.69	0	-0.05
53.	Camphene	-2.31	-1.82	0	0.03
54.	(-)-delta-selinene	-2.27	-1.69	0	0.01
55.	α-humulene	-1.02	-0.92	0	0.02
56.	Decanal	-0.6	-1.68	-1.33	-0.4

It has been seen that the Capsaicin, Dihydrocapsaicin, Santalol, Farnesol, (-)-delta-selinene, Myrtenal, trans-geraniol, E)-Nerolidol, Eugenol, a-bisabolol, Citronellol, Thujones, a-thujone, Sabinol, Epicurzerenone, Terpinen-4-ol, trans-chrysanthenyl acetate, gama-terpinene, Decanal, Geranial, Bornyl acetate, (E)-cinnamaldehyde, neral, cis-ß-guaiene, methyl cinnamate, beta pinenoxid, Borneol, Myristicin, ß-phellandrene, terpenyl acetate, globulol, ascaridole, caryophyllene oxide, Santolina alcohol, Pregeijerene B had high binding affinity for the NFκB receptor than the selective inhibitor, parthenolide (Figure 5). The dock score of the capsaicin molecule with the receptor site of NFκB was found to be -6.33kcal/ mol which shows higher binding affinity of the capsaicin molecule for the receptor site as compared to the selective inhibitors parthenolide whose dock score was -3.04kcal/ mol. Capsaicin molecule interacts with the receptor site showing hydrogen bond interaction with the water molecule present on the receptor site and hydrophobic interaction with the VAL 414, ALA 427, LEU 471, LEU472, LEU 522 and CYS533 amino acid residues of the receptor site (Figure 5b). Capsaicin also shows electrostatic forces of attraction with the GLU 413, ASP 534, ASP 519, ARG 416, ARG 408, ASP 519 amino acid residues of the receptor site. These interaction profiles are responsible for the highest binding affinity which this molecule shows with the receptor site. It has been reported by S Singh et al., that when capsaicin was tested on human myeloid ML-1a cells capsaicin blocked TNF-mediated activation of NFκB in a dose- and time-dependent manner [22]. The results of docking of the molecules with the NFκB receptor are depicted in Table 4.

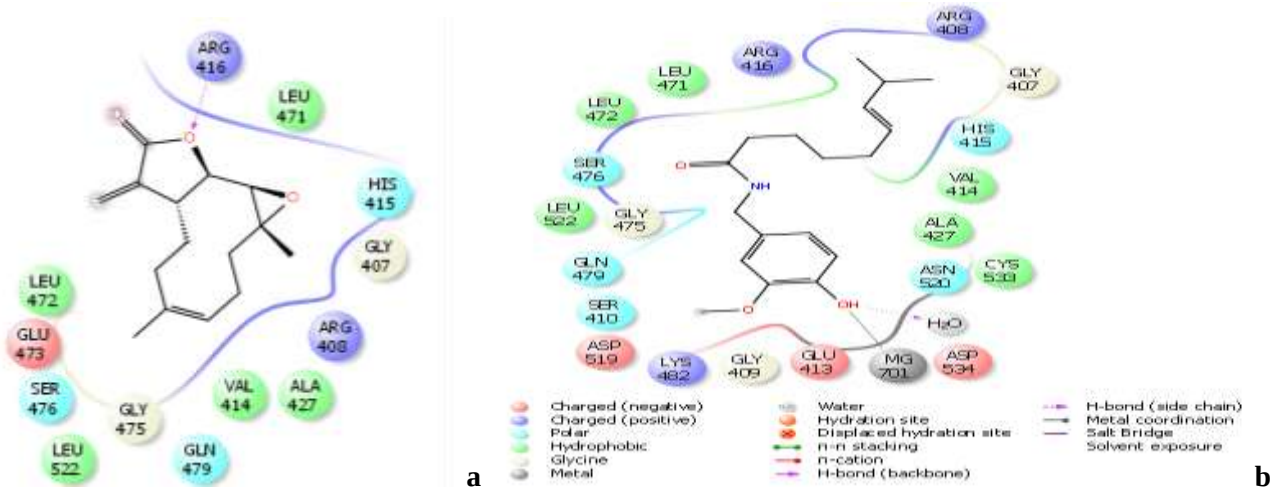

Figure 5: Interaction profile and docked poses of Parthenolide (a) and Capsaicin with NFκB receptor (b).

Table 4: Docking scores of essential oil molecule with NFκB receptor

S. No.	Ligands	Dock Score	Lipophilic Evdw	H Bond	Electro
1.	Capsaicin	-6.33	-4.14	-0.96	-0.74
2.	dihydrocapsaicin	-5.97	-3.6	-1.11	-0.77
3.	Santalol	-5.32	-3.74	-0.7	-0.38
4.	Farnesol	-5.12	-3.24	-0.69	-0.7
5.	(-)-delta-selinene	-4.87	-4.43	0	0.06
6.	Myrtenal	-4.75	-2.44	-0.7	-0.11
7.	trans-geraniol	-4.75	-2.81	-0.7	-0.73
8.	E)-Nerolidol	-4.74	-3.7	-0.49	-0.05
9.	Eugenol	-4.69	-2.53	-0.96	-0.7
10.	α-bisabolol	-4.46	-3.73	-0.68	-0.13
11.	Citronellol	-4.43	-2.51	-0.54	-0.88
12.	Thujones	-4.37	-2.29	-0.59	-0.17
13.	α-thujone	-4.37	-2.29	-0.59	-0.17
14.	Sabinol	-4.36	-2.46	-0.98	-0.42
15.	Terpinen-4-ol	-4.34	-2.95	-0.7	-0.19
16.	trans-chrysanthenyl acetate	-3.93	-2.74	-0.69	-0.19
17.	γ-terpinene	-3.83	-3.36	0	0.02
18.	Decanal	-3.83	-2.39	-0.25	-0.69
19.	Geranial	-3.7	-2.68	0	-0.52
20.	(E)-cinnamaldehyde	-3.62	-2.25	-0.59	-0.27
21.	neral	-3.62	-2.19	-0.25	-0.68
22.	cis-ß-guaiene	-3.54	-3.05	0	0.02
23.	methyl cinnamate	-3.49	-2.4	-0.7	-0.13
24.	Borneol	-3.45	-1.96	-0.7	-0.29
25.	Myristicin	-3.44	-2.36	-0.75	-0.21
26.	ß-phellandrene	-3.44	-2.97	0	0.03
27.	terpenyl acetate	-3.43	-2.21	-0.86	-0.17
28.	globulol	-3.36	-2.87	0	0.01
29.	ascaridole	-3.31	-2.38	-0.35	-0.08
30.	caryophyllene oxide	-3.26	-2.34	-0.35	-0.08
31.	Santolina alcohol	-3.26	-2.57	-0.71	-0.28
32.	Pregeijerene B	-3.26	-2.75	0	-0.01
33.	α-cadinol	-3.23	-1.94	-0.7	-0.09
34.	α-bisabolol oxide B	-3.19	-3.01	-0.63	-0.31
35.	α-thujene	-3.18	-2.67	0	-0.01
36.	thymoquinone	-3.16	-2.28	-0.19	-0.2
37.	α- pinene	-3.1	-2.63	0	0.02
38.	Parthenolide(Inhibitor)	-3.04	-2.01	-0.43	-0.1
39.	humulene epoxide	-3.02	-2.52	0	0
40.	aromadendrene	-3.01	-2.58	0	0.07
41.	α - muurolol	-2.99	-1.71	-0.75	-0.03
42.	Valancene	-2.99	-2.72	0	0.06

S. No.	Ligands	Dock Score	Lipophilic Evdw	H Bond	Electro
43.	α-gurjunene	-2.98	-2.48	0	0
44.	Sabinene	-2.98	-2.47	0	0
45.	(+)-delta-Cadinene	-2.96	-2.48	0	0.03
46.	10_epi_eudesmol	-2.93	-2.03	-0.43	-0.12
47.	7-epi-a-eudesmol	-2.92	-1.77	-0.7	-0.09
48.	Camphene	-2.89	-2.41	0	0.02
49.	p-cymene	-2.85	-2.36	0	0.01
50.	β-elemene	-2.77	-2.85	0	0.07
51.	ß-selinene	-2.71	-2.34	0	0
52.	pregeijerene	-2.67	-2.23	0	0.06
53.	methyl chavicol	-2.54	-1.94	-0.53	-0.14
54.	Myrcene	-2.37	-3.16	0	-0.02
55.	α-humulene	-2.33	-1.91	0	0.09
56.	β-myrcene	-2.32	-3.12	0	-0.01

Since the photochemical bind efficiently on the NFκB receptor so tried to dock these molecules on the traf 6 and ikk-β which are involved in the signaling pathway of NFκB. Interaction profile of ikk with capsaicin is depicted in Figure 6a. Interaction profile of traf 6 with dihydrocapsaicin is depicted in Figure 6b.

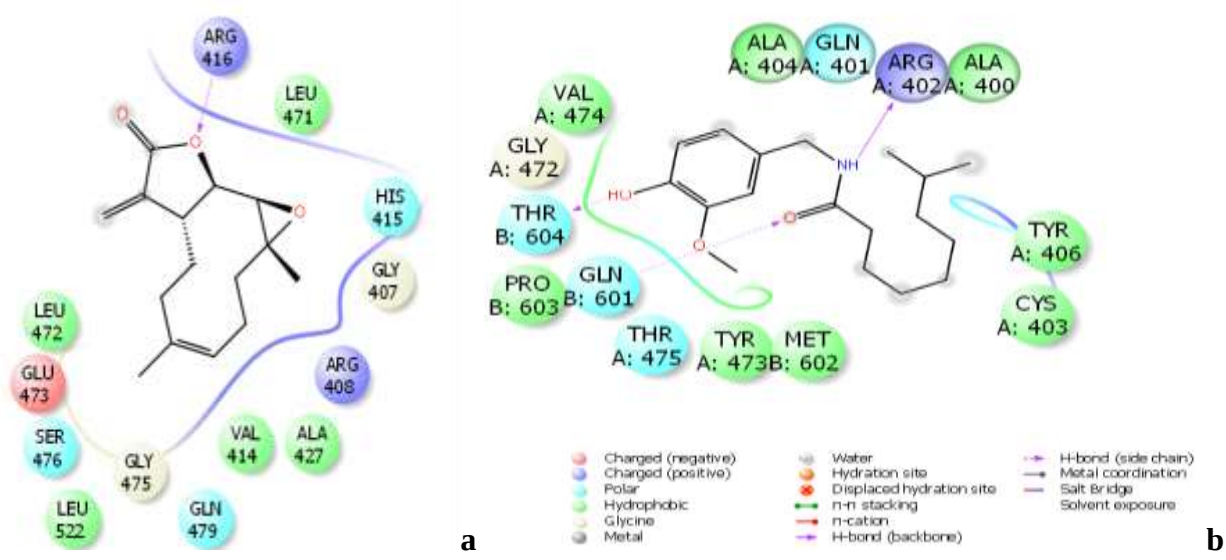

Figure 6: Interaction profile and docked poses of (a) and capsaicin with ikk beta receptor capsaicin with traf 6 (b).

Conclusion

To summarize, we have employed virtual screening protocol, molecular docking to identify potential drug-like inhibitors of the apoptotic signaling pathway. Several potential drug-like inhibitors have been screened and found to interact with traf-6, ikk beta, NF kappa B and BCL-2 anti-apoptotic proteins including BCL2, BCL-xl and MCL-1 satisfactorily.

Phytochemicals like Capsaicin, Dihydrocapsaicin, (±) delta-C adinene revealed strong binding with less docking scores to BCL-2 anti-apoptotic protein as compared with the selective inhibitor of the BCL-2 protein i.e., ABT-263. Santalol, dihydrocapsaicin, a-bisabolol oxide B, Capsaicin Showed comparable binding affinity for BCL-xl receptor but less than that of the selective inhibitor of BCL-xl. Whereas the photochemical Capsaicin and Dihydrocapsaicin had low affinity for MCL-1 receptor than the selective inhibitor obatoclax with a little difference in the dock score. It has been seen that the Capsaicin, Dihydro-capsaicin, Santalol, Farnesol, (-)-delta-selinene, Myrtenal, trans-geraniol, E)-Nerolidol, Eugenol, a-bisabolol, Citronellol, Thujones, a-thujone, Sabinol, Epicurzerenone, Terpinen-4-ol, trans-chrysanthenyl acetate, gama-terpinene, Decanal, Geranial, Bornyl

acetate, (E)-cinnamaldehyde, neral, cis-ß-guaiene, methyl cinnamate, beta pinenoxid, Borneol, Myristicin, ß-phellandrene, terpenyl acetate, globulol, ascaridole, caryophyllene oxide, Santolina alcohol, Pregeijerene-B had high binding affinity for the NF kappa B receptor than the selective inhibitor i.e., parthenolide. This *in-silico* docking study validates the anti apoptotic properties of these naturally occurring compounds hence these compounds can be considered as alternative drug therapy, of natural origin, for treatment of cancer through inhibition of NF kappa-B and BCL-2 anti-apoptotic family proteins. Further studies are required to make them as lead compounds for the development of novel drugs against cancer.

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