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Research Article

Screening of Novel CXC Chemokine Receptor 4 Inhibitors from Ethyl Acetate Extract of *Alpinia purpurata* Using GC-MS Analysis and its Molecular Docking Studies

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ABSTRACT

CXCR4 is the receptor for a chemokine, CXCL12 (stromal cell-derived factor-1, SDF-1), it has been proven to be involved in several problematic diseases, including AIDS, cancer cell metastasis, leukemia cell progression and rheumatoid arthritis. Hence it's thought to be an important therapeutic target to overcome of these diseases. *Alpinia purpurata* belongs to the family of Zingiberaceae and it possesses many biological activities. Therefore, the aim of present study is to identify the novel CXCR4 inhibitors from the bioactive compounds present in ethyl acetate extract of *Alpinia purpurata* using GC-MS analysis and its molecular docking studies. Results, GC-MS analysis shown that, ethyl acetate extract of *Alpinia purpurata* contain 32 bioactive compounds. Molecular docking studies of theses bioactive compounds (Glide 5.5 from Schrödinger suite) revealed that, out of 32 bioactive compounds, 1,2,3-trimethyl-5-propan-2-ylbenzene, Isocamphane, 1,10-Dimethyl-2-methylene-trans-decalin,1-Bromoeicosane,2-(2-Isopropenyl-5-ethylcyclopentylmethoxy) tetrahydropyran, Dihexadecylphosphate, Naphthalene, 1,1'-(1,2-ethanediyl)bis[decahydro- shows the better glide score compared with Cyclophophamide (FDA approved drug). ADME properties (Qikprop 2.3 from Schrödinger suite) of these bioactive compounds were under the acceptable range. Based on the result it can be concluded that, these bioactive compounds may act as novel inhibitors for CXCR4. In future it may focus on current discoveries in CXCR4 inhibition.

Keywords: CXCR4, *A. purpurata*, GC-MS, Docking analysis, ADME properties.

INTRODUCTION

The chemokine receptor CXCR4 belongs to the large super family of G protein-coupled receptors and has been identified to play a crucial role in a number of biological processes, including the trafficking and homeostasis of immune cells such as T lymphocytes[1].CXCR4 has also been found to be a prognostic marker in various types of cancer, including leukemia and breast cancer, and recent evidence has highlighted the role of CXCR4 in prostate cancer[2]. Several independent studies have shown that cancer cells have higher levels of CXCR4 expression compared to normal cells[3].

In recent years the use of plants in the management and treatment of diseases has gained considerable importance. Plants and fruits are considered one of the main sources of biologically active compounds an estimate of the world health organization (WHO) states that around 85-90% of the world's population consumes traditional herbal medicines[4].Bioactive compounds from medicinal plants with anticancer and anti-inflammatory effects have become key resources in drug discovery fields for the treatment of various malignancies and immunological disorders [5]. A huge reservoir of bioactive compounds exists in many species of plants of Earth, only a small percentage of which have been examined and continued to be an important source of anticancer agents. Worldwide effects are ongoing to identify new anticancer compounds from plants. With the current decline in the number of new molecular entities from the pharmaceutical industry, novel anticancer agents are being sought from traditional medicines[6]. *Alpinia* is the largest genus of the family with more than 200 species[7]. Many *Alpinia* species are well-known medicinal herbs that have been shown by several previous studies to have various effects, namely, anti-inflammatory[8], antioxidant, antimicrobial[9].Rhizome has sharp odour, improves appetite, taste and voice. It is also used for headache, rheumatism, sore throat and renal disease[10]. *Alpinia purpurata* is the medicinal plant belongs to the family of Zingiberaceae, it constituents promote antimicrobial activity against certain microorganisms[11].In addition to the purported anti-inflammatory activity, its phytomedicinal potential to treat tuberculosis is also described[12]. Antidermatophytic[13], antinociceptive[14], hepatoprotective[15], immunostimulatory [16], and anticancer[17], activities. *Alpinia purpurata* may serve as

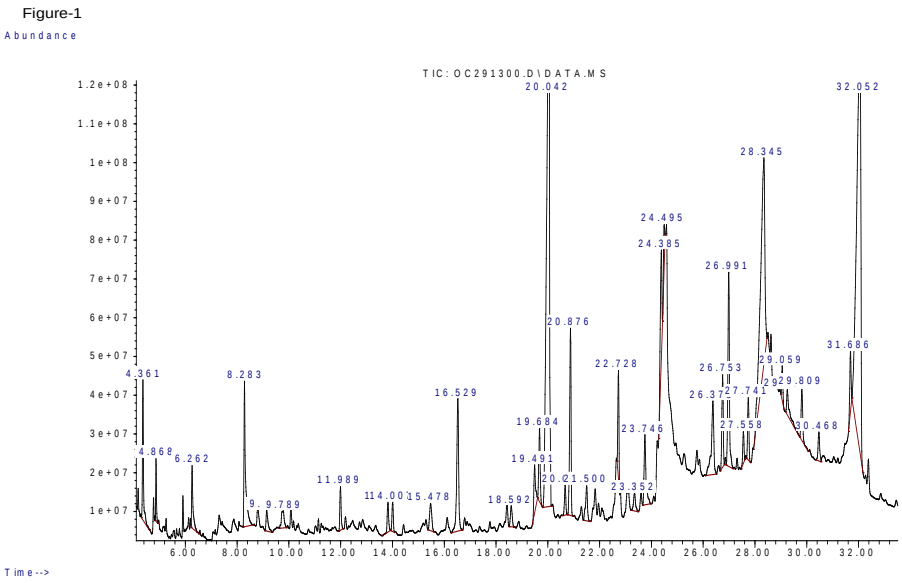


Fig.1. GC-MS peak level in the chromatogram graph of ethyl acetate extract of *Alpinia purpurata*

potential antioxidant and anticancer agents against ovarian cancer cell lines[18]. Phytochemical studies on *Alpinia purpurata* revealed that it possess flavonoids, rutin, kaempferol-3-rutinoside and kaempferol-3-oliucronide[19]. Rutin had the highest concentration in both extracts. This flavonoid is widely distributed in the Plant Kingdom and, despite its hydrophilic character; it presents several therapeutic applications such as antioxidant activity, besides reducing arteriosclerosis risks and increasing vein tone, which improves the blood flow[20]. Therefore, objective of the study is to analyze the presence of bioactive compounds from ethyl acetate extract of *Alpinia purpurata* using GC-MS analysis and to screen the novel inhibitors for CXCR4 from the identified bioactive compounds by molecular docking studies.

Table 1. Gas chromatogram graph peak level of ethyl acetate extract of *Alpinia purpurata*

Peak	RT	Compound Name	%
1	4.361	1,2,3-Trimethyl-5-propan-2-ylbenzene.1	1.81
2	4.868	1-Hexanol, 2-ethyl-	0.63
3	6.262	1H-Pyrazole, 4,5-dihydro-5,5-dimethyl-4-isopropylidene-	1.05
4	8.283	2-Decenal, (E)-	2.99
5	9.141	2,4-Decadienal	0.53
6	9.789	2,4-Diisopropenyl-1,1dimethylcyclohexane.1	0.63
7	11.989	Isocamphane.1	0.71

Table 1. Gas chromatogram graph peak level of ethyl acetate extract of *Alpinia purpurata*

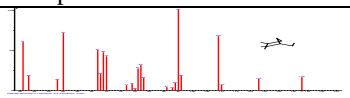
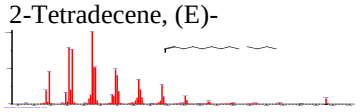
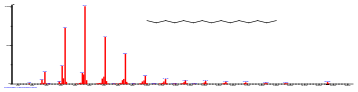
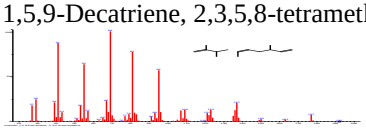
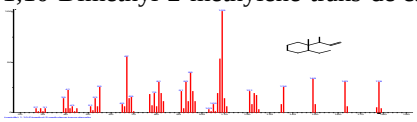
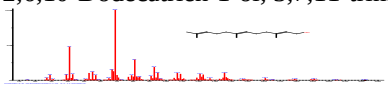
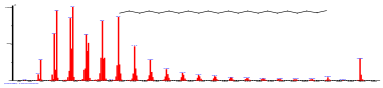
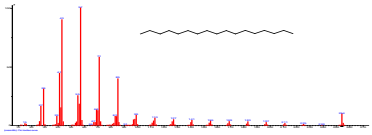
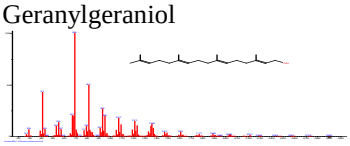
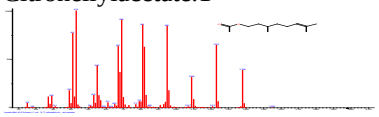
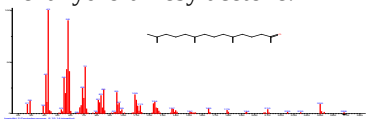
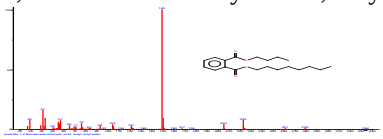
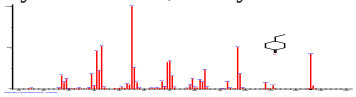
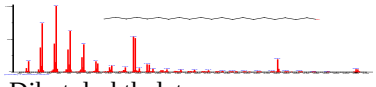
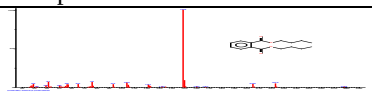
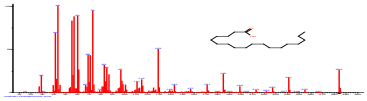
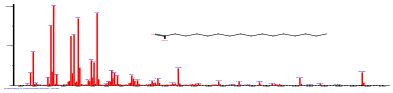
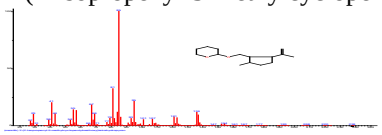
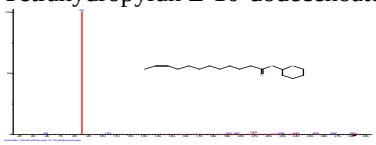
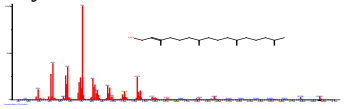
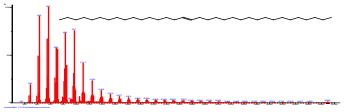
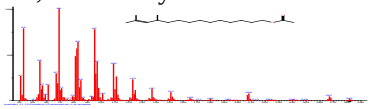
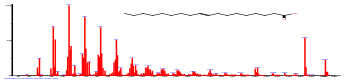
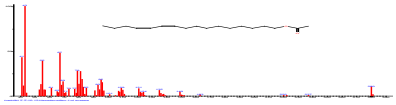
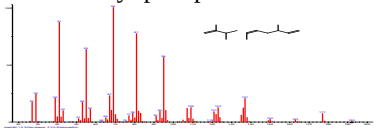
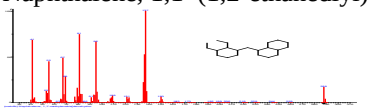
Peak	RT	Compound Name	%
			
8	13.825	2-Tetradecene, (E)-	0.56
			
9	14.007	Hexadecane	0.5
			
10	15.478	1,5,9-Decatriene, 2,3,5,8-tetramethyl-	0.76
			
11	16.529	1,10-Dimethyl-2-methylene-trans-de calin	3.35
			
12	18.592	2,6,10-Dodecatrien-1-ol, 3,7,11-trimethyl-	0.47
			
13	19.491	1-Docosene	1.08
			
14	19.684	Octadecane	1.33
			
15	20.042	Geranylgeraniol	22.24
			
16	20.666	Citronellylacetate.1	0.51
			
17	20.876	Hexahydrofarnesylacetone.1	3.39
			
18	21.5	1,2-Benzenedicarboxylic acid, butyl octyl ester	0.86
			
19	22.728	Cyclohexanone, 4-ethyl-	1.46
			
20	23.352	1-Bromoeicosane	0.46
			
21	23.746	Dibutyl phthalate	1.8

Table 1. Gas chromatogram graph peak level of ethyl acetate extract of *Alpinia purpurata*

Peak	RT	Compound Name	%
			
22	24.385	Octadecanoic acid	2.48
			
23	24.495	n-Hexadecanoic acid	0.77
			
24	26.378	2-(2-Isopropenyl-5-methylcyclopentylmethoxy)tetrahydropyran	2.41
			
25	26.753	Tetrahydropyran Z-10-dodecenoate	1.65
			
26	26.991	Phytol	3.87
			
27	27.558	17-Pentatriacontene	0.52
			
28	29.059	11,13-Dimethyl-12-tetradecen-1-ol acetate	0.5
			
29	29.251	Elaidic acid.1	0.75
			
30	30.468	Z,Z-10,12-Hexadecadien-1-ol acetat	0.67
			
31	31.686	Dihexadecyl phosphate	1.07
			
32	32.052	Naphthalene, 1,1'-(1,2-ethanediyl)bis[decahydro-	22.95
			

MATERIALS AND METHODS

Plant collection

Alpinia purpurata was collected from Kanyakumari, Tamil Nadu, India. The plant specimen was authenticated by Dr. G.V.S. Murthy, Botanical Survey of India, Coimbatore, TNAU campus, India. A voucher specimen has been deposited in the laboratory for future reference (BSI/SC/5/23/10-11/Tech) [18].

Preparation of extract

The leaf of *Alpinia purpurata* were washed thoroughly in tap water, shade dried and powdered. The powder (100 g) was exhaustively extracted with ethyl acetate in the ratio of 1:5 for 24 h by using soxhlet apparatus. The extract

Table 2. Docking results of CXCR4 protein complex with 32 bioactive compounds from GC-MS analysis of ethyl acetate extract of *Alpinia purpurata*

S. No	Compound Name	Glide Score	Glide energy
1.	1,2,3-Trimethyl-5-propan-2-ylbenzene.1	-6.137	-16.422
2.	1-Hexanol.1	-1.696	-16.655
3.	1H-Pyrazole, 4,5-dihydro-5,5-dimethyl-4-isopropylidene-.1	-3.487	-15.769
4.	2-Decenal.1	1.554	-22.889
5.	2,4-Decadienal.1	-3.258	-18.817
6.	2,4-Diisopropenyl-1,1-dimethylcyclohexane.1	-4.978	-8.878
7.	Isocamphane.1	-5.180	-7.151
8.	(2Z)-2-Tetradecene.1	0.097	-23.413
9.	Hexadecane.1	-0.346	-27.092
10.	1,5,9-Decatriene, 2,3,5,8-tetramethyl-.1	-2.133	-22.387
11.	1,10-Dimethyl-2-methylene-trans-decalin.1	-5.701	-6.780
12.	2,6,10-Dodecatrien-1-ol, 3,7,11-trimethyl-.1	-2.921	-28.024
13.	1-Docosene.1	-4.641	-29.787
14.	Octadecane.1	-1.035	-26.073
15.	Geranylgeraniol.1	-3.556	-30.328
16.	Citronellyl acetate.1	-1.026	-23.344
17.	Hexahydrofarnesyl acetone.1	-2.735	-24.047
18.	Butyl octyl phthalate.1	-3.905	-33.539
19.	4-Ethyl-3,4-dimethylcyclohexanone.1	-4.275	-16.424
20.	1-Bromoeicosane.1	-5.603	-34.382
21.	Dibutyl phthalate.1	-2.185	-34.258
22.	Octadecanoic acid.1	-4.069	-33.617
23.	n-Hexadecanoic acid.1	-4.093	-32.775
24.	2-(2-Isopropenyl-5-methylcyclopentylmethoxy)tetrahydropyran.1	-5.357	-18.319
25.	Tetrahydropyran Z-10-dodecenoate.1	-2.174	-32.637
26.	Phytol.1	-3.891	-30.248
27.	17-Pentatriacontene.1	-2.831	-35.998
28.	11,13-Dimethyl-12-tetradecen-1-ol acetate.1	-2.428	-33.873
29.	Elaidic acid.1	-3.353	-36.192
30.	Z,Z-10,12-Hexadecadien-1-ol acetate.1	-2.643	-35.249
31.	Dihexadecyl phosphate.1	-6.414	-52.244
32.	Naphthalene, 1,1'-(1,2-ethanediyl)bis[decahydro-.1	-5.107	-13.693
33	Cyclophosphamide	-5.040	-30.861

was completely evaporated to dryness using rotary flash evaporator (Buchi type). The ethyl acetate extract of the plant was used for GC-MS analysis.

GC-MS analysis

GC-MS analysis of the whole plant extract of *Alpinia purpurata* was performed using the equipment Agilent technologies 7890 A. The equipment has a DB 35 – MS Capillary Standard non-polar column with dimensions of 30 mm×0.25 mm ID×0.25 µm film. The carrier gas used was Helium with at low of 1.0 ml/min. The injector was operated at 250 °C and the oven temperature was programmed as follows: 60 °C for 15 min, then gradually increased to 280 °C at 3 min. The identification of components was based on Willey and NIST libraries as well as comparison of their retention indices. The constituents were identified after comparison with those available in the computer library (NIST and Willey) attached to the GC-MS instrument and the results obtained have been tabulated.

In Silico Analysis

Preparation of protein structure

The 3D structure of CXCR4 was retrieved from the Protein Data Bank (PDB ID: 3OE6) and it was prepared by protein preparation wizards (standard methods) that are available in grid-based ligand docking with energetics[21]. Protein was optimized using sample water orientation and minimized by using RMSD 0.30 Å and OPLS (2005) force field.

Active site prediction

The active site (binding pockets) and functional residues of CXCR4 was identified and characterized by Site- Map module from Schrodinger package. Site Map calculation begins with an initial search step that identifies or characterizes- through the use of grid points- one or more regions on the protein surface that may be suitable for binding ligands to the receptor. Contour maps were then generated, produced hydrophobic, hydrophilic maps hydrogen binding possibilities which may guide the protein- ligand docking analysis [22].

Ligand preparation

Totally 32 bioactive compounds (from GC-MS analysis) were used in molecular docking studies. These ligands

Table 3. ADME properties of screened novel CXCR4 inhibitors

S. No	Ligands	Molecular Weight (g/mol)	H-Bond Donor	H-Bond Acceptor	Log *P* O/W
1.	1,2,3-Trimethyl-5-propan-2-ylbenzene.1	162.274	0	0	4.713
2.	Isocamphane.1	138.252	0	0	4.759
3.	1,10-Dimethyl-2-methylene-trans-decalin.1	178.317	0	0	4.376
4.	1-Bromoeicosane.1	361.448	0	0	11.567
5.	2-(2-Isopropenyl-5-methylcyclopentylmethoxy) tetrahydropyran.1	238.369	0	3.4	2.641
6.	Dihexadecyl phosphate.1	546.853	1	5	11.232
7.	Naphthalene, 1,1'-(1,2-ethanediyl)bis[decahydro-.1	330.596	0	0	10.3
8.	Cyclophosphamide	261.087	1	8.5	0.854

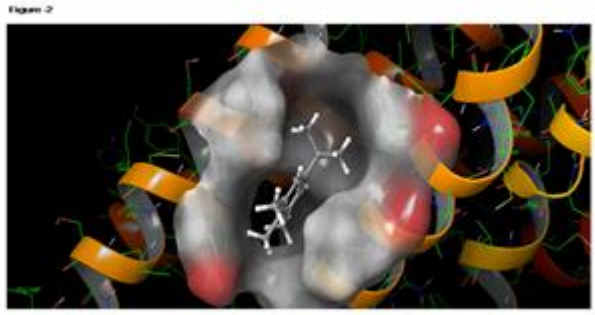


Fig.2. The bioactive compound of 1, 2, 3-trimethyl-5-propan-2-yl benzene complex with CXCR4

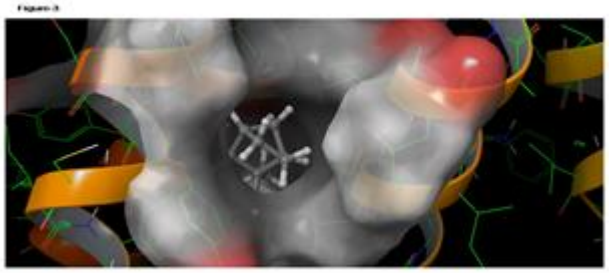


Fig.3. The bioactive compound of Isocamphane complex with CXCR4.

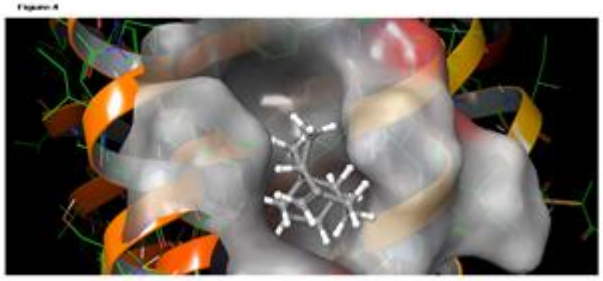


Fig.4. The bioactive compound of 1, 10-Dimethyl-2-methylene-trans-decalin complex with CXCR4.

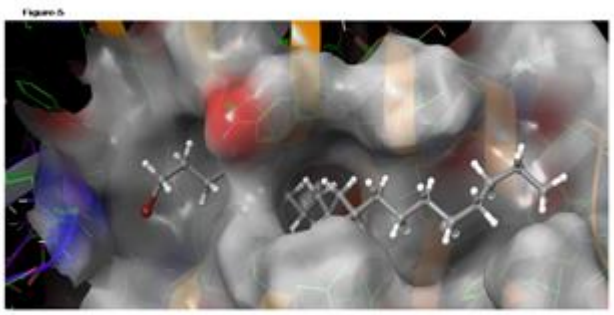


Fig. 5. The bioactive compound of 1-Bromoeicosane, complex with CXCR4

were prepared using the LigPrep 2.4. The structure of each ligands were optimized by means of the OPLS 2005 force field using a default setting [23].

Molecular docking analysis

All docking analysis were performed by using the standard precision (SP) which is Standard mode of Glide 5.6 (Grid-based Ligand Docking with Energetic) module from Schrodinger 2012. All bioactive compounds were docked in to the binding site CXCR4 receptor using Glide 5.6. The scaling Vander Waals radii were 1.0 in the receptor grid generation. Grid was prepared with the bounding box set on 20Aº. The co-ordinates of this enclosing box with the help of the active site residues to be set default. The force field is using for the docking protocol is OPLS_2005. The lowest-energy docked complexes were found in the majority of similar docking conformations[24].

ADME properties prediction

The CXCR4 inhibitors were checked for their ADME properties using QikProp 2.3 module. QikProp helps in analyzing the pharmacokinetics and pharmacodynamics of the ligand by accessing the drug like properties. Predicted significant ADME properties such as Molecular weight (MW), H-Bond donor, H-Bond acceptor and log *P* (O/W)[25].

RESULTS AND DISCUSSION

CXCR4 is the predominant chemokine receptor in ovarian cancer and has also been implicated in many other cancers including prostate, colon and ovary. Recent studies have suggested that malignant cells use chemokine receptor/ligand interactions to home in on common metastatic sites like bone marrow and the lungs. In some cancers, over-expression of CXCR4 has been observed to lead to metastasis[26]. This particular interaction is vital in early embryonic development as it is

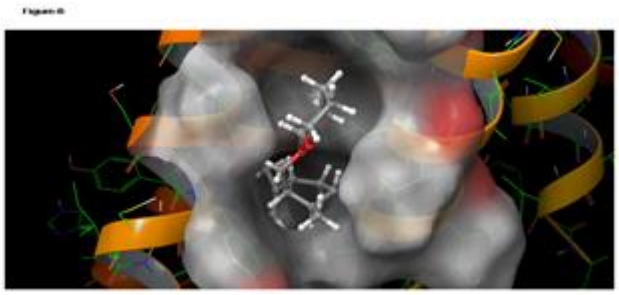


Fig. 6. The bioactive compound of 2-(2-Isopropenyl-5-methylcyclopentylmethoxy) tetrahydropyran complex with CXCR4

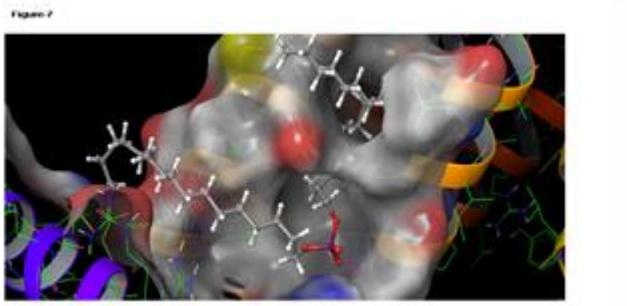


Fig.7. The bioactive compound of Dihexadecyl phosphate complex with CXCR4

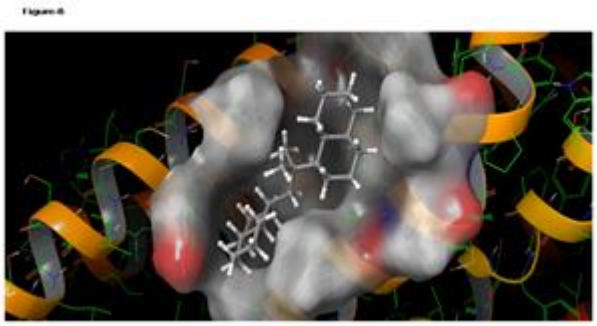


Fig.8. The bioactive compound of Naphthalene, 1, 1'-(1, 2-ethanediyl)bis[decahydro-Cyclopentadecane complex with CXCR4

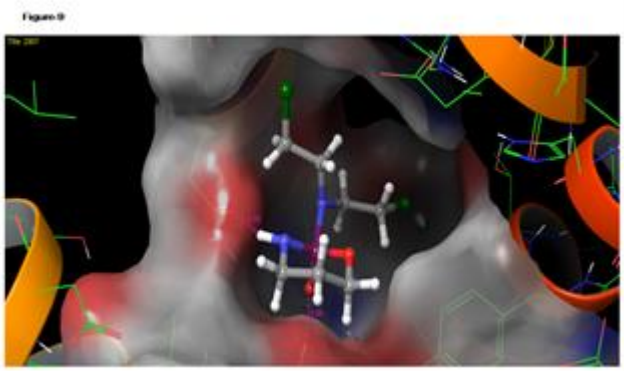


Fig. 9. The bioactive compound of Cyclophosphamide complex with CXCR4

required for the correct formation of vascular, nervous, hematopoietic and cardiac systems[27].Faults in the CXCR4/CXCL12 interaction during the embryonic stage can lead to several defects including cardiac dysfunction and bone marrow defects.

Herbal treatment proves its efficacy in medicinal field without any side effects as synthetic medicines have; hence it's preferred to be more beneficial. Plant extracts as well as plant-derived compounds are found to be excellent medicinal agents for several cancers.

GC-MS analysis shows that, ethyl acetate extract of *Alpinia purpurata* has 32 bioactive compounds. A peak level in the chromatogram graph indicates the maximum amount of Naphthalene, 1, 1'-(1, 2-ethanediyl) bis [decahydro- (22.94 %) present in the extract was showed in figure 1 and table1.

These bioactive compounds posses many biological activities such as antioxidant, anticancer, anti-inflammatory, hypocholesterolemic, antiarthritic, antimicrobial, antidiabetic activity etc., Octadecadienoic acid (Z, Z) have the property of anti inflammatory, hypocholesterolemic and antiarthritic activity which was reported by the earlier workers [28, 29]. Naphthalene also having good antimicrobial activity[30].Geranylgeranyl pyrophosphate is an intermediate in the HMG-CoA reductase pathway used by organisms in the biosynthesis of terpenes and terpenoids. In plants it is also the precursor to carotenoids, gibberellins, tocopherols, and chlorophylls. Geranylgeraniol had a broader protective effect against the cytotoxicity of statins than exogenous ubiquinone. Therefore, geranylgeraniol may be a more useful and practical means of limiting the toxicities of Statins, without reducing their efficacy as cholesterol lowering agents[31]. Phytol is one part of the cholorophyll and important in plant biosynthesis. When human as well as rodents are fed free phytol a high proportion is absorbed and converted into phytonic acid. The phytol conversion of phytonic acid is a natural rexinoid it shows antidiabetic activity in type II Diabetic patients[32]. Hexadeconoic acid has the property of antioxidant and antimicrobial activities[33]. All cholorophyll derivatives are having antioxidant properties[34]. N-hexadecane has also been used as a Substrate for the bacterial production of biosurfactants[35]. N-Hexadecane has been utilized in model studies of the transmembrane domain (TMD) of G protein, as related to the kinetics of poly (ethylene glycol) (PEG)-mediated fusion of small unilamellar vesicles[36].

The CXCR4 protein was retrieved (from PDB ID: 3OE6) and prepared for further studies. On the other hand, the bioactive compounds (from *Alpinia purpurata*) were prepared. The best active site (binding pocket/site) was preferred based on the site score and hydrophobic/hydrophilic areas, which holds better binding cavity. The binding site residues of PPARγ were predicted and it may involve in the binding of substrate and small molecule. Thus, all these residues were confirmed as CXCR4 active site residues and picked to generate grid in the centroid of these residues for molecular docking approach. The molecular docking is

frequently used to predict the binding orientation of small molecule drug candidate to their protein targets in order to predict the affinity and activity of the small molecule. The docking results revealed that,32 bioactive compounds were complexes with CXCR4 protein shown in table 2.Among these bioactive compounds,1,2,3-trimethyl-5-propan-2-ylbenzene,Isocamphane,1,10-Dimethyl-2-methylene-trans-decalin,1-Bromoeicosane,2-(2-Isopropenyl-5-methylcyclopentylmethoxy)tetrahydropyran,Dihexadecyl phosphate,Naphthalene, 1,1'-(1,2-ethanediyl)bis[decahydro- shows the better glide score of -6.137,-5.180,-5.701,5.603,-5.357,-6.414,-5.107 respectively when compared with FDA approved drug of cyclophosphamide glide score -5.040. These bioactive compounds complexes with CXCR4 were shown in figure 2 to 9. The highest negative value of glide score has been indicated that, these complexes may have good affinity and it may have the inhibitory activity against CXCR4. The ADME properties prediction results (shown in table 3) of these bioactive compounds were under acceptable range.

CONCLUSION

The medicinal plant of *Alpinia purpurata* (ethyl acetate extract) posses 32 bioactive compounds were identified by GC-MS analysis. These bioactive compounds have been reported to have much biological activity against variety of human diseases. The molecular docking studies exposed that, out of these bioactive compounds1,2,3-trimethyl-5-propan-2-ylbenzene, Isocamphane, 1,10-Dimethyl-2-methylene-trans-decalin, 1-Bromoeicosane, 2-(2-Isopropenyl-5-methylcyclopentylmethoxy) tetrahydropyran, Dihexadecyl phosphate, Naphthalene, 1,1'-(1,2-ethanediyl)bis[decahydro- shows the better glide score compared with Cyclophophamide (FDA approved drug). ADME properties of these bioactive compounds were under the acceptable range. Therefore it can be concluded that, these bioactive compounds may act as novel inhibitors for CXCR4. In future, these compounds may lead to identify their potential biological activities by *in vitro* and *in vivo* studies.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this paper.

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