J Young Pharm, 2018; 10(2): 173-177
A multifaceted peer reviewed journal in the field of Pharmacy
www.jyoungpharm.org | www.phcog.net

Original Article

Analysis of novel C-X-C chemokine receptor type 4 (CXCR4) inhibitors from hexane extract of *Euclea crispa* (Thunb.) leaves by chemical fingerprint identification and molecular docking analysis

Chella Perumal Palanisamy and Anofi Omotayo Tom Ashafa*
Phytomedicine and Phytopharmacology Research Group, Department of Plant Sciences, University of the Free State, Qwaqwa Campus,Phuthaditjhaba, SOUTH AFRICA.

ABSTRACT

Objectives: To evaluate the potential inhibitors aginst CXCR4 from hexane extract of *Euclea crispa* (*E. crispa*) leaves. **Methods:** The natural compounds were identified and charecterized through GC-MS analysis. Molecular docking studies carried out to investigatethe inhibitory activity angainst CXCR4.Absorption, Distribution, Metabolism, and Excretion (ADME) properties also were predicted to find pharmacokinetics and pharmacodynamics of compounds.**Results:**The molecular docking simulations revealed that, Benzoic acid 3-methyl-4-(1,3,3,3-tetrafluoro-2-methoxycarbonyl-propenylsulfanyl)-phenyl ester (SCHEMBL15979821), Hydrocortisone Acetate and Phenyl Glucuronide possess good inhibitory activites(Glide Score of -7.06, -6.97, -6.47 and Glide Energy of -43.22, -48.27 and -32.80 kcal/mol respectively) when compared with standard FDA approved drug and other compounds. All the screened compounds were within the acceptable and permissible limits of ADME properties. **Conclusion:** Thus, from this study it canbe concluded that, these screened natural compounds from *E. crispa* leaves may serve as potential inhibitors for CXCR4 and they might leads to development of new therapeutic agents against cancer and its associated complications.

Key words: *E.crispa,* GC-MS analysis, CXCR4, Molecular docking, ADME properties.

Correspondence:

Dr. AOT Ashafa, Department of Plant Sciences, University of the Free State, Qwaqwa Campus, Phuthaditjhaba 9866 South Africa.

Phone: +27-587185313

Email: ashafaaot@ufs.ac.za

DOI: 10.5530/jyp.2018.10.39

INTRODUCTION

Chemokine gradients cells direct themigration of cells during many physiological and pathologic processes. Cancer cells express chemokine receptors which respond to chemokine gradients resolving in the growth and spread of cancer.[1] The chemokine receptor 4 (CXCR4) is a G-protein-coupled membrane receptor that is expressed by a majority of cancer types.[2] CXCR4 has been shown to play a critical role in cancer progression and metastatic spread. It has also been reported that 69% of ductal carcinoma *in situ* (DCIS) lesions are CXCR4-positive. Overexpression of CXCR4 has also been suggested to be of prognostic value for imaging applications and diagnosis.[3] Emerging reviews highlight-CXCR4 as a target in cancer metastasis and HIV.[4]

Natural products are structurally diverse and have more potential than synthetic compounds. Moreover, they are the source of most of the active ingredients in medicines.[5] *E. crispa* is an afro-tropical plant species, commonly known as the blue guarri (Eng.); bloughwarrie (Afr.); motlhaletsogane (Setswana) and iDungamuzi, umGwali (isiZulu). It is hardy and evergreen plants that usually forms a dense stand of shrubs, or grow to tree size. It is widespread and common in the interior regions of southern Africa.[6] This plant is used traditionally against wide range of ailments such as gonorrhea, leprosy, scabies, diarrhea and wound infections and previous reports have shown that the plant possessed anti-bacterial and antifungal activities.[7] Virtual screening based on computer-aided techniques are very promising in drug discovery and plays an important role in identifying lead compounds from natural products.[8] Therefore, the aim of the present study is to screen the novel CXCR4 inhibitors from *E. crispa* leaf extract to delineate the mechanism behind its anticancer activities.

MATERIALS AND METHOD

Plant collection

Fresh leaves of *E. crispa* were collected from Qwaqwa campus, University of the Free State, South Africa during the month of April 2017 and identified by Prof. AOT Ashafa. The plant sample was authenticated at University of the Free State herbarium with herbarium collection of Taylor and Van Wyk, 1994 with reference number: 6404000-400. Collected plant leaves were washed under running tap water to remove contaminants and foliar debris, air dried, powdered and stored in air tight container at 4°C for further studies.

Preparation of extract

Using exhaustive extraction procedure, the powdered plant material (100 g) was soaked with hexane (500 ml) and kept on the shaker (Labcon Platform Shaker, PTY, Durban, South Africa) for 72 hours at room temperature. The extract was collected, filtered using Whatman No:1 filter paper and concentrated to dryness using rotary evaporator (Cole-Palmer, South Africa) set at 40°C. The dried extracts were stored at 4°C until further use.

GC-MS analysis

GC-MS analysis of hexane extract of *E. crispa* was done using Agilent technologies 7890 A (DB 35 – MS Capillary Standard non-polar column with dimensions of 30 mm×0.25 mm ID×0.25 µm film). Helium was used as carrier gas at low down of 1.0 ml/minutes. The injector was functioned at 250°C and oven heat was maintained as follows: 60°C for 15 minutes, then slowly amplified to 280°C at 3 minutes. MS were taken at 70 eV; a scan distance of 0.5 seconds and fragments starts from 50 to 650 Da. Total GC operation periods was 25 minutes. The comparative percentage amount of every module was calculated by evaluating its average peak area to the total areas, software adopted to handle mass spectra and chromatograms was Turbo mass. The percentage composition of compounds in the plant extract was calculated. Interpretation of GC-MS was done by the National Institute Standard and Technology (NIST) database and Willey libraries in addition to comparison of their retention indices.[9]

Computational molecular analysis

Ligand selection and preparation

The natural compounds were selected from GC-MS analyzedhexane extract of *E. crispa* and FDA approved drug of Cyclophosphamide (standard drug for comparison) also were prepared using the LigPrep (LigPrep, Schrödinger, LLC, New York, NY, 2017) for molecular docking analysis. The structure of each ligands were optimized by means of the OPLS 2005 force field using a default setting.

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 (Protein Preparation Wizard; Epik, Schrödinger, LLC, New York, NY, 2017). 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 were identified and characterized using SiteMap (SiteMap, Schrödinger, LLC, New York, NY, 2017). SiteMap 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, hydrogen binding possibilities, hydrophilic maps, produced hydrophobic are may guide the protein- ligand docking analysis.

Molecular docking analysis

All docking analysis were performed using the standard precision (SP) which is Standard mode of Glide (Glide, Schrödinger, LLC, New York, NY, 2017) a grid based ligand docking with energetic. All selected natural compounds were docked in to the binding site of CXCR4 using Glide modiule. The scaling Vander Waals radii were 1.0 in the receptor grid generation. Grid was prepared with the bounding box set on 20A°. The co-ordinates of this enclosing box with the help of the active site residues to be set as default. The force field used for the docking protocol was OPLS_2005. The docked lowest-energy complexes were found in the majority of similar docking conformations.

ADME properties prediction

The CXCR4 ligands identified from *E. crispa* leaf extract were checked for their ADME properties using QikProp (QikProp, Schrödinger, LLC, New York, NY, 2017). It helps to analyze the pharmacokinetics and pharmacodynamics of the ligands by accessing the drug like properties. The significant ADME properties such as Molecular weight (MW), H-Bond donor, HBond acceptor and log P (O/W) were predicted.

RESULTS

The GC-MS analysis characterized variety of compounds (Figure 1) from *E. crispa* leaves extract (retention time, percentage of composition, molecular formula, molecular mass and their peak area were given in Table 1.) The compound prediction is based on NIST library and it showed 29 compounds namely; Tetracosane (14.98%), Dodecane (10.76%), 2-Ethyl-1-decanol (8.00%), Tridecane (7.53%), 4,5,6,8-PTetramethoxy-2,3-dihydroindeno[1,2,3-ij]isoquinolin-9-ol (6.99%), Diphenylvinylphosphine (6.38%), Squalene (5.85%), Triacontane (5.27%), 2,6-Dimethylheptadecane (5.02%), Docosane (3.68%), Tetradecane (3.59%), 1-Hepten-3-ol (2.63%), Orthotolidine (2.31%), Phenyl Glucuronide (2.25%), 5-tridecylbenzene-1,3-diol (1.90%), Benzoic acid 3-methyl-4-(1,3,3,3-tetrafluoro-2-methoxycarb onyl-propenylsulfanyl)-phenyl ester (1.76%), Pentadecane (1.68%), 6-(4,6-DIOXO-1,4,5,6-Tetrahydropyrimidin-2-YL-amino) hexanoic acid trifluoroacetate (1.22%), Benzhydrazide,3-chloro-N2-[3-(4-methoxyphenyl)-1-methyl-3-oxopropenyl- (1.09%), Hydrocortisone Acetate (0.95%), Triacontane (0.95%), Dioctyl phthalate (0.78%), Phytol (0.66%), Shogaol (0.49%), 1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-hexadecamethyloctasiloxane (0.47%), Docosane (0.44%), Tetradecamethyl hexasiloxane (0.42%), 3-(4-Chlorophenyl)-5-styryl[1,2,4]oxadiazole (0.32%), Ephedrine (0.32%).

In molecular docking analysis, 29 identified natural compounds and FDA approved drug of Cyclophosphamide were complexed with CXCR4 protein shown in Table 2. Among the complexes CXCR4/SCHEMBL15979821 (Figure 2A), CXCR4/Hydrocortisone Acetate (Figure 2B) and CXCR4/Phenyl Glucuronide (Figure 2C) complexes showed good affinity (Glide Score of -7.06, -6.97, -6.47 and Glide Energy of -43.22, -48.27 and -32.80 kcal/mol respectively) than CXCR4/Cyclophosphamide Complex (Glide Score of -3.50 and Glide Energy of -26.56) showed in Figure 2D. These screened compounds strongly predicted to bind hydrophobic region of CXCR4 protein.

The ADME properties prediction of screened compounds (SCHEMBL15979821, Hydrocortisone Acetate and Phenyl)stating these parameters were under acceptable and permissible range which suggests that *E. crispa* leaves could be further explored for biopropecting of cheap, safe and affordable anticancer drug for the continent of Africa and the world at large.

DISCUSSION

A large proportion of the world population depends upon traditional medicine because of the shortage and high expenses of orthodox medicine. Comprehension of the chemical constituents of medicinal plant is helpful in the discovery of therapeutic agents as well as new sources of economic materials like oil and gums. Secondary metabolites of medicinal plants have proved to be an excellent reservoir of new medicinal compounds.[10] Phytochemicals are primary and secondary compounds. Chlorophyll, proteins and common sugars are included in primary constituents and secondary compounds have terpenoid, alkaloids and phenolic compounds. Alkaloids and terpenoids exhibit various important pharmacological activities i.e., anti-inflammatory, anticancer, anti-malarial, inhibition of cholesterol synthesis, anti-viral and anti-bacterial activities. Terpenoids are very important in attracting useful mites and consume the herbivorous insects. Alkaloids are used as anaesthetic agents and are found in medicinal plants.[11] Variety of bioactive compounds is present in medicinal plants and they are widely used against various diseases. The demand for natural food constituents has resulted in broad research on naturally occurring bioactive compounds which are able to develop novel drug agents for many diseases.[12] In

Table 1: GC-MS chromatograph reported the retention time and percentage the composition of the identified bioactive compounds from hexane leaf extract of *E. crispa*

S. No	RT	Compounds	Molecular formula	Molecular Weight	Peak Area (%)
1	4.11	Hydrocortisone Acetate	C23H32O6	404	0.95
2	4.92	Docosane	C22H46	310	3.68
3	5.89	1-Hepten-3-ol	C7H14O	114	2.63
4	6.40	2-Ethyl-1-decanol	C12H26O	186	8.00
5	7.37	4,5,6,8-PTetramethoxy-2,3-dihydroindeno[1,2, 3-ij]isoquinolin-9-ol	C19H19NO5	341	6.99
6	7.87	Dodecane	C12H26	170	10.76
7	8.87	2,6-Dimethylheptadecane	C19H40	268	5.02
8	9.28	Tridecane	C13H28	184	7.53
9	10.02	Tetradecane	C14H30	198	3.59
10	10.38	Benzoic acid 3-methyl-4-(1,3,3,3-tetrafluoro-2-methoxycarbonyl-propenylsulfanyl)-phenyl ester	C19H14F4O4S	414	1.76
11	11.83	6-(4,6-DIOXO-1,4,5,6-TETRAHYDROPYRIMIDIN-2-YL-AMINO)HEXANOIC ACID TRIFLUOROACETATE	C12H16F3N3O6	355	1.22
12	12.88	Pentadecane	C15H32	212	1.68
13	14.98	3-(4-Chlorophenyl)-5-styryl[1,2,4]oxadiazole	C16H11ClN2O	182	0.32
14	15.36	Benzhydrazide,3-chloro-N2-[3-(4-methoxyphenyl)-1-methyl-3-oxopropenyl-	C18H17ClN2O3	344	1.09
15	16.69	Orthotolidine	C14H16N2	212	2.31
16	17.67	Diphenylvinylphosphine	C14H13P	212	6.38
17	19.69	1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-hexadecamethyloctasiloxane	C16H50O7Si8	579	0.47
18	19.98	Phytol	C20H40O	296	0.66
19	21.39	Shogaol	C17H24O3	276	0.49
20	21.77	Phenyl Glucuronide	C12H14O7	270	2.25
21	25.12	5-tridecylbenzene-1,3-diol	C19H32O2	292	1.90
22	29.43	Ephedrine	C10H15NO	165	0.32
23	30.70	Tetradecamethyl hexasiloxane	C14H42O5Si6	458	0.42
24	31.10	Triacontane	C30H62	422	0.95
25	31.48	Dioctyl phthalate	C24H38O4	390	0.78
26	32.46	Docosane	C22H46	310	0.44
27	34.03	Triacontane	C30H62	422	5.27
28	36.22	Squalene	C30H50	410	5.85
29	38.46	Tetracosane	C24H50	338	14.98

Table 2: ADME properties of screened natural compounds and Cyclophosphamide as predicted.

S. No	Ligands	Molecular Weight (g/mol)	H-Bond donor	H-Bond acceptor	LogP O/W
1	SCHEMBL15979821	414.370	0	9	4.6
2	Hydrocortisone Acetate	404.503	2	6	2.2
3	Phenyl Glucuronide	270.237	4	7	1.4
4	Cyclophosphamide	261.082	1	4	0.60

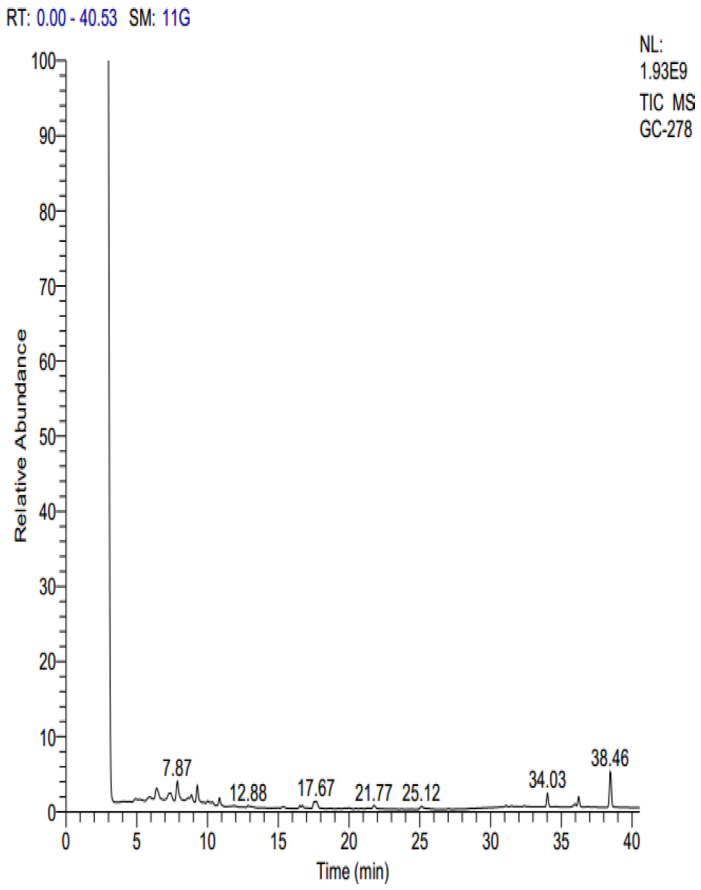

Figure 1: GC-MS chromatogram of hexane extract of *E. crispa* leaves

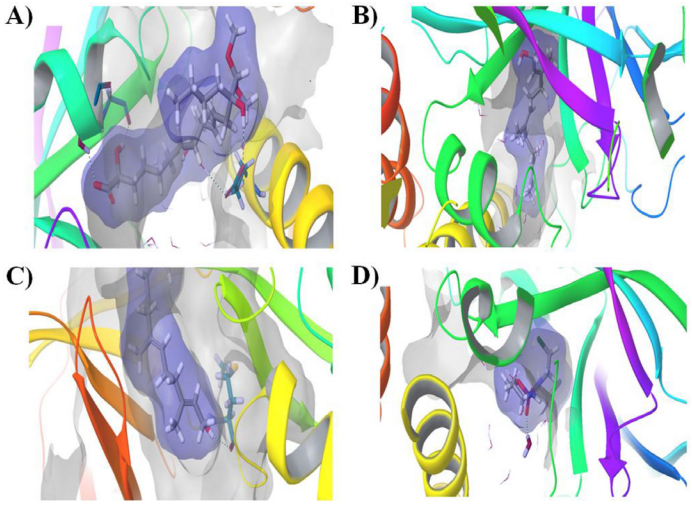

Figure 2: Docking complexes of CXCR4 with A) SCHEMBL15979821, B) Hydrocortisone Acetate, C) Phenyl Glucuronide and D) Cyclophosphamide.

South Africa, great number of plant species had been screened for their pharmacological properties but still a vast wealth of rare species is yet to be unexplored. Characterization of phytochemicals by chromatography and spectroscopic methods could identify and deliver the efficient information of herbal medicines.[13] GC-MS is a combined analytical method that utilizes the features of gas-liquid chromatography and mass spectrometry to recognize variety natural compounds present in the plant extract.[14] In this study, GC-MS analysis was characterized variety of compounds were present the ethanolic extract of *E. crispa* and peak level in the chromatogram graph indicates the maximum amount of Tetracosane (14.98%), Dodecane (10.76%), 2-Ethyl-1-decanol (8.00%), Tridecane (7.53%), 4,5,6,8-PTetramethoxy-2,3-dihydroindeno[1,2,3-ij] isoquinolin-9-ol (6.99%), Diphenylvinylphosphine (6.38%), Squalene (5.85%), Triacontane (5.27%), 2,6-Dimethylheptadecane (5.02%) were present in the extract. These bioactive compounds could posses many biological activities against human disease management system.[15]

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 and this approach has been used in modern drug design to understand drug-receptor interactions. In addition, it can be used to know the mechanism of drug-receptor interactions. It was performed in order to characterize the compounds on the basis of their ability to form favorable interactions within the active site of protein[16] The best active site (binding pocket/site) was determined based on the site score and hydrophobic/hydrophilic areas, which holds better binding cavity.[17] The binding site residues of CXCR4 were predicted and the results from this study showed that it may involve in the binding of substrate and small molecule. Thus, CXCR4 active site residues were picked to generate grid in the centroid of these residues for molecular docking approach.[18] Among the identifed 25 bioactive compounds from the *E. crispa* leaves extract, SCHEMBL15979821, Hydrocortisone Acetat and Phenyl Glucuronide has better binding affinity with CXCR4 when compared with other compounds and these compounds may lead to develop an novel therapeutic agents.

ADME properties of screened compounds do not predict any adverse effect that could be implicated in the failure of drugs. Consequently, there is increasing awareness in the early prediction of ADME properties reaching success rate of compounds development with the objectives.[19] The limitations of ADME properties are: not more than 5 hydrogen bond donors, not more than 10 hydrogen bond acceptors, molecular mass less than 500 daltons, an octanol- water partition coefficient log P not greater than 5. These screened compounds were within the acceptable and permissible limits of ADME properties.

CONCLUSION

The present study identified 29 natural compounds from hexane extract of *E. crispa* which hold many biological (state few in bracket) activities. The molecular docking studies revealed that, out of these bioactive compounds, SCHEMBL15979821, Hydrocortisone Acetate and Phenyl Glucuronide showed better interaction with CXCR4 protein. The ADME properties prediction of these compounds was under acceptable range. Based on the results from this study it can be concluded that, these bioactive compounds may act as novel inhibitors for CXCR4 protein. However, further studies are warranted to evaluate the findings of present study. Nevertheless, the present study reports the presence of promising phytoconstituents that could inhibit the activity of CXCR4.

ACKNOWLEDGEMENT

The authors are thankful to the Directorate Research Development of the University of the Free State,South Africa for the postdoctoral fellowship award to Dr Chella Perumal Palanisamy and Alagappa University, Karaikudi, Tamil Nadu, India for providing facilities and encouragement to complete this research work.

CONFLICT OF INTEREST

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

REFERENCES

1. Barbieri F, Bajetto A, Florio T. Role of Chemokine Network in the Development and Progression of Ovarian Cancer: A Potential Novel Pharmacological Target. J Oncology. 2010;2010:1-15.
2. Sun X, Cheng G, Hao M, Zheng J, Zhou X, Zhang J, *et al*. CXCL12/CXCR4/CXCR7 Chemokine Axis and Cancer Progression. Cancer Metastasis Rev. 2010; 29(4): 709-22.
3. Kuil J, Yuan H, Buckle T, Oishi S, Fujii N, Josephson L, *et al*. Synthesis and *in vitro* evaluation of a bimodal CXCR4 antagonistic peptide. Bioconjug Chem. 2011;22(5):859-64.
4. Busillo JM, Benovic JL. Regulation of CXCR4 signaling. Biochim Biophys Acta. 2007;1768(4):952-63.
5. Egieyeh S, Syce J, Christoffels A, Malan SF. Exploration of Scaffolds from Natural Products with Antiplasmodial Activities, Currently Registered Antimalarial Drugs and Public Malarial Screen Data. Molecules. 2016;21(104):1-14.
6. Alayande KA, Pohl CH, Ashafa AOT. Time-kill kinetics and biocidal effect of *Euclea crispa* leaf extracts against microbial membrane. Asian Pac J Trop Med. 2017;10(4):390-9.
7. Alayande KA, Pohl CH, Ashafa TOM. Assessment of antidiarrhoea properties of *Euclea crispa* (Thunb.) leaf extract and fractions. S Afr J Bot. 2016;103:306-6.
8. Pretorius JC, Magama S, Zietsman P. Purification and identification of antibacterial compounds from *Euclea crispa* subsp. crispa (Ebenaceae) leaves. S Afr J Bot. 2003;69(4):579-86.
9. Perumal PC, Sowmya S, Pratibha P, Vidya B, Anusooriya P, Starlin T, *et al*. Identification of novel PPARγ agonist from GC-MS analysis of ethanolic extract of *Cayratia trifolia* (L.): a computational molecular simulation studies. J App Pharm Sci. 2014;4:06-11.
10. Priyanga S, Hemmalakshmi S, Vidya B, Perumal PC, Gopalakrishnan VK, Devaki K. Acute toxicity assessment of ethanolic leaf extract of *Macrotyloma uniflorum* L. on Wistar albino rats. Toxicology Int. 2017; 24(1): 22-27.
11. Perumal PC, Sophia D, Raj CA, Ragavendran P, Starlin T, Gopalakrishnan VK. *In vitro* antioxidant activities and HPTLC analysis of ethanolic extract of *Cayratia trifolia* (L.). Asian Pac J Trop Dis. 2012; 2: S952-S956.
12. Perumal PC, Sowmya S, Pratibha P, Vidya B, Anusooriya P, Starlin T, *et al*. Isolation, structural characterization and in silico drug-like properties prediction of bioactive compound from ethanolic extract of *Cayratia trifolia* (L.). Pharmacog Res. 2015;7:121-5.
13. Pratibha P, Sophia D, Perumal PC, Gopalakrishnan VK. *In-silico* docking analysis of *Emilia sonchifolia* (l.) dc. gas chromatography-mass spectroscopy derived terpenoid compounds against pancreatic cancer targets (AKT and BRCA2). World J Pharm Pharm Sci. 2014;3:1844-55.
14. Ghosh G, Panda P, Rath M, Pal A, Sharma T, Das D. GC-MS analysis of bioactive compounds in the methanol extract of *Clerodendrum viscosum* leaves. Pharmacognosy Res. 2015;7(1):110-3.
15. Rahman MM, Habib MR, Hasan MA, Al Amin M, Saha A, Mannan A. Comparative assessment on *in vitro* antioxidant activities of ethanol extracts of *Averrhoa bilimbi*, *Gymnema sylvestre* and *Capsicum frutescens*. Pharmacognosy Research. 2014;6(1):36-41.
16. Tripathi SK, Singh SK, Singh P, Chellaperumal P, Reddy KK, Selvaraj C. Exploring the selectivity of a ligand complex with CDK2/CDK1: a molecular dynamics simulation approach. J Mol Recognit. 2012;25:504-12.
17. Perumal PC, Sowmya S, Velmurugan D, Sivaraman T, Gopalakrishnan VK. Assessment of dual inhibitory activity of isolated natural compound (Epifriedelanol) from *Cayratia trifolia* (L.) against ovarian cancer. Bangladesh J Pharmacol. 2016;11:545-51.
18. Srinivasan P, Perumal PC, Sudha A. Discovery of Novel Inhibitors for Nek6 Protein through Homology Model Assisted Structure Based Virtual Screening and Molecular Docking Approaches. Scientific World J. 2014;2014:1-9.
19. Butina D, Segall MD, Frankcombe K. Predicting ADME properties *in silico*: methods and models. Drug. Discov Today. 2002;7(11):S83-8.

Article History: Submission Date : 05-10-2017 ; Revised Date : 02-11-2017; Acceptance Date : 12-11-2017.
Cite this article: Palanisamy CP, Ashafa AOT. Analysis of novel C-X-C chemokine receptor type 4 (CXCR4) inhibitors from hexane extract of *Euclea crispa* (Thunb.) leaves by Chemical fingerprint identification and molecular docking analysis. J Young Pharm. 2018;10(2):173-7.