Original Research Article **Bioinformatics**

International Journal of Pharma and Bio Sciences **ISSN 0975-6299**

MOLECULAR DOCKING AND DFT STUDIES OF COMPOUNDS IDENTIFIED FROM *BARLERIA CRISTATA* LEAVES TO RHEUMATOID ARTHRITIS PROTEIN

J. IRSHAD AHAMED[1], ABIRAMI KANDHASWAMY[1], K. S. MEENA*[1]

[1]Bioinformatics Infrastructure Facility Center, Queen Mary's College, University of Madras, Chennai – 600004, Tamil Nadu, India

ABSTRACT

Barleria plant of family Acanthaceae is used traditionally for a wide variety of biomedical properties such as anemia, toothache, cough, hypoglycemic agent, anti inflammatory agent. Owing to the multiple pharmacological properties, the compounds were investigated for the treatment of autoimmune disorders by *in silico* approach. Phytochemical analysis, spectral characterization by GC-MS followed by Molecular Docking of the characterized compound were performed. ADMET and DFT studies were carried out to select the suitability of drug compounds. Totally nineteen compounds were obtained and depending upon the highest mass percentage five compounds were selected. i) 6-Amino-1,2,3,4tetrahydroindan-5,7-Dione ii) 9, 12, 15 – Octadecatrienoicacid, methyl ester, iii) 1,2-Benzenedicarboxylic acid, butyl 2-methyl propyl ester iv) p-Decylphenoxsy acetic acid and v) 2,6,6-Trimethyl-2-3-[3-Oxo -3 – phenylpropenyl) cyclohexane for further studies. The selected five compounds qualified the Lipinski rule of five and were non – mutagenic and non – carcinogenic. Thus the compound p-Decylphenoxy acetic acid was found to be a potent and safe against HLA-DR4 associated with rheumatoid arthritis.

KEYWORDS: ***Barleria cristata*, GC-MS, Molecular Docking, Rheumatoid Arthritis**

K. S. MEENA*

Bioinformatics Infrastructure Facility Center, Queen Mary's College, University of Madras, Chennai – 600004, Tamil Nadu, India

***Corresponding Author**

Received on: 05-01-2017
Revised and Accepted on: 24-03-2017
DOI: http://dx.doi.org/10.22376/ijpbs.2017.8.2.b562-571

INTRODUCTION

Barleria is a genus of plants belonging to the family Acanthaceae. The species *Barleria* consists of 28 taxa, including 26 genus and one sub-genus. It is the third largest species in the family Acanthaceae with 300 species[1-2]. *Barleria cristata* is commonly known as Philippine violet. Blue bell *Barleria* or Crested Philippine violet is cultivated as an ornamental plant in villages and gardens. The plants are 6-10 meter tall, leaves are dark green on upper surface, lower surface pale green, elliptic to narrowly ovate, 2.5-10 cm long, flowers are spinymargined, the inner 2-lobes linear, 7 mm long, entire margins, corolla violet, pink or white, funnel forms 5 - 5.7 cm long.[3] The biological investigation of the plant showed anti-inflammatory, anti-anemic and anti-toothache, anti-plasmodial, antioxidant properties. Previous phytochemical studies with the plant led to the isolation and structure elucidation of flavonoids, phenolic compounds, iridoidal and phenylethanoid glycosides.[4,5,6,7] *Barleria crisata* has been used as a traditional herbal therapy in Thailand and it allegedly acts as a tonic, diuretic and blood purifier.[8] The plant is used in skin diseases, bronchitis, blood diseases and asthma.[9] The plants of the genus *Barleria* are used for boils, bee bite, tooth ache.[10] The earlier phytochemical study of the Aerial parts of *Barleria cristata* L. Deals with the isolation and structure elucidation of iridoid and flavonoid glycosides.[11] Rheumatoid arthritis is an autoimmune disorder and the association of human leukocyte antigen (HLA) with RA was reported in 1970, in specific, with a set of alleles of the HLA - DRB1 gene locus. Also HLA – DRB 1 alleles (DRB1*04:01 and DRB1 *04:04) has accounted for RA in Caucasians ethnic group as proven by serological evidences. Thus the prime association of HLADRB4 with RA emphasize the development of novel drugs for treatment. Hence the compounds from Barleria plant were explored against the target 1D5X (HLADR4) and the screened compounds were subjected further for *in silico* ADMET(Absorption, Distribution, Metabolism, Excretion, Toxicity) & DFT(Density Functional Theory studies).

MATERIALS AND METHODS

Extraction of plant

Leaf of *Barleria cristata* was thoroughly washed and air dried under shade for four days. The dried leaves were finely powdered. 50ml solvents of ethyl acetate, ethanol, chloroform and 10gm of plant sample was taken in 250ml conical flask for a magnetic stirring method for 48 hours. The extracts were filtered through whatman filter paper 40 pore size. The crude was discarded and the pure liquid extract was collected. The extracts were stored at 4ºC for future use.

Phytochemical analysis

The alkaloids were determined by Wagner,s test[12], carbohydrate by Benedict's test[12], saponin by foam test, phenols by ferric chloride test, flavonoids by lead acetate test, diterpenes by copper acetate test, terpenoids by Salwoski's test, aminoacids by ninhydrin test, protein by biuret test, Oxalate by ethanoic acid glacial, xanthoproteins by conc, HNO_3 test, cardiac glycosides by kellerkillani synthesis, anthocynanin by HCl and NH_3, leucoxanthanin by isoamyl alcohol, carboxylic acid by effervescence test.

GC –MS analysis

GC-MS analysis was carried out on a GC clarus 500 Perkin Elmer system comprising a AOC-20i autosampler and gas chromatograph interfaced to a mass spectrometer instrument employing the following environment: column Elite-1 fused silica capillary column (30 x 0.25mm ID x 1µMdf, composed of 100% Dimethyl polydiloxane), operating in electron impact mode at 70eV; Helium gas (99.999%) was used as carrier gas at a constant flow of 1 ml /min and an injection volume of 0.5 µl was worked(split ratio of 10:1) injector temperature 250 ºC; ion-source temperature 280 ºC. The oven temperature was set at 110 ºC (isothermal for 2 min), with an increase of 10 ºC/min, to 200ºC, then 5ºC/min to 280ºC, ending with a 9min isothermal at 280ºC. Mass spectra were taken at 70eV; a scan interval of 0.5 seconds and fragments from 40 to 450 Da. Total GC running time is 36min. The relative percentage amount of each component was calculated by comparing its average peak area to the total areas. Software adopted to handle mass spectra and chromatograms was a Turbo Mass Ver 5.2.0.

Molecular docking

Molecular docking was carried out to shed light on the binding modalities of investigating ligands towards its target HLA-DR4 (PDB code: 1D5X). Its X- ray diffraction resolution of 2.45 Å.The molecular docking program of Flexible Docking studies was utilized to decide the potential binding mode between the compound and the selected target HLA-DR4. The preparation of retrieved protein was executed using the prepare protein wizard of DS4.0 by applying CHARMm force field. Initially, all the internal legend, water molecules, ions and metal element were removed and inserted the missing atom before minimization of target protein. The prepared target was further used for docking analysis**.** The active site analysis of the receptor protein 1D5X was predicted using Discovery studio 4.0 based on 'Receptor cavity method'. The amino acids responsible for binding the diverse ligands are seen in these binding regions. Based on this protocol active site of the target receptor were obtained and they were chosen for docking analysis.

Ligand Docking and Scoring

Prepared ligand and receptor were used as an initial coordinates for the docking purpose. We have used HLA-DR4 complexed with the dipeptide mimetic and Seb as the target receptor. The principle ligand can be docked by two methods: i) Assuming that the ligand is flexible and receptor is rigid and ii) Assuming that the ligand is rigid and receptor is flexible. So here we have used both strategies of ligand docking. In both processes we have used flexible docking method. During the first docking process, the receptor was treated as flexed while ligand was flexible. After docking, the results were used for calculating with respectively C-Docker energy, libdock score, binding energy.

(ADMET) Evaluation of drug likeliness and Toxicity prediction

The drug likeliness of the selected active phytochemicals was analyzed using Lipinski's and Veber rules. The rules are molecular weight < 500 Daltons, number of hydrogen bond donors <5 and the number of hydrogen bond acceptors < 10. The ligands passing the Lipinski properties were taken for docking studies.

DFT Computational Quantum Chemical Calculation Studies

DFT calculations were performed in this present study with the local function of PWC, program (Material studio $DMol^3$ Version 6.1). The input parameter was selected from DS4.0, DFT and $DMol^3$ properties of total energy, binding energy, (HOMO) energy, (LUMO) energy, dipole Magnetic, (ESP) charge, band gap energy, dipole compound, Mulliken charge, hirshfeid charge. DFT energy was performed by single – point calculation and geometry optimization were searched for a minimum energy structure.

RESULTS

Phytochemical studies

The total phytochemical constituent of *Berlaria cristata* is given in (Table-1 and Table-2). The phytochemical constituents are rich in alkaloid, cardiac glycosides, saponin, xanthoproteins and diterpenes. Analysis of *Barleria cristata* leaf extract with solvent of ethyl acetate is more abundant in saponins, diterpenes, cardiac glycosides, xanthoproteins and lesser in alkaloids, oxalate. Ethanol extracts were more abundant in diterpenes, cardiac glycosides, xanthoproteins and lesser in saponins, flavnoids, oxalate. Chloroform extract more abundant in alkaloids, saponins, diterpenes, carboxylic acid and lesser in Cardiac glycosides and Xanthoproteins. Flavonoids exhibit several biological effects such as anti-inflammatory, anti-fungal, anti-hepatotoxic and anti-ulcer actions.[22] The phytochemical characters of the *Barleria cristata* leaves were investigated. The qualitative phytochemical analysis of ethanol, ethyl acetate, chloroform extract of *Barleria cristata* leaves extract contains flavonoids, saponin, terpenoids, steroids, alkaloids, polyphenols and tannin which important in disease prevention.

Table 1
Phytochemical screening of various solvent extracts from leaves Barleriya cristata (L.)

Phytochemical Test	References	Leaves		
		Name of the Solvents Used		
		Ethyl Acetate	Ethanol	Chloroform
Alkaloids (Wagner's Test)	Prashant Tiwari et al., 2011. [12]	+	_	+++
Carbohydrates (Benedict's test)	Prashant Tiwari et al., 2011. [12]	_	_	_
Saponins (Foam Test)	Prashant Tiwari et al., 2011. [12]	+++	+	+++
Phenols (Ferric chloride)	Prashant Tiwari et al., 2011. [12]	_	_	_
Flavonids (Lead Acetate)	Prashant Tiwari et al., 2011. [12]	_	+	_
Amino acids (Ninhydrin Test)	Prashant Tiwari et al., 2011. [12]	_	_	_
Diterpenes (Copper acetate)	Prashant Tiwari et al., 2011. [12]	++	++	+++
Terpenoids (Salwoski's Test)	Zakia Khanam et al., 2014. [17]	_	_	_
Protein (Biuret Test)	Prashant Tiwari et al., 2011. [12]	_	_	_
Oxalate (Ethanolic acid glacial)	Solomon Charles Ugochukwu et al., 2013. [18]	+	+	_
Cardiac glycosides (Kellar Kiliani synthesis)	Chandra Shekar Misa et al., 2011 [19]	++	++	+
Anthocyanin (Hcl and NH_3)	Ashvin Godghate et al., 2012 [20]	_	_	_
Leucoanthocyanin (Isoamyl alcohol)	Ashvin Godghate et al., 2012 [20]	_	_	_
Carboxylic acid (Effervescence Test)	Sumam Kumar et al., 2013.[21]	_	_	++
Xanthoproteins (Conc.HNO_3, _NH_3	Sumam Kumar et al., 2013.[21]	+++	+++	+

Ea – Ethyl Acetate, Et – Ethanol, Ch – Chloroform.
+++ Strongly Present, ++ moderately Present, +Weekly Present, - Absent. *The given Results are Statistically Significant.

Table 2
Barleria cristata leaf of phytochemical content with different extract

Solvents	Leaf
Ethyl acetate	Alkaloids, saponins, diterpenes, oxalate, Cardiac glycosides, xanthoproteins.
Ethanol	Saponins, flavonoids, dierpenes, oxalate, Cardiac glycosides, xanthoproteins.
Chloroform	Alkaloids, saponins, diterpenes, cardiac glycosides, carboxylic acid, xanthoproteins.

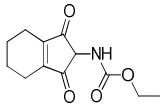
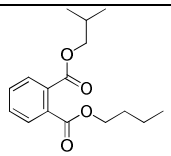
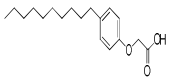
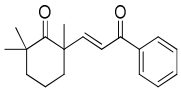
GC-MS Analysis

In GC-MS analysis nineteen compounds were identified from ethanolic extract, chloroform extract, ethyl acetate extract of the plant leaf of *Barleria cristata* by Gas Chromatogram- Mass spectrometry (GC-MS) analysis results. The active principles with their molecular formula, molecular weight (MW) and concentration (%) are presented of selected five higher m/z percentage compounds was given in (Table 3). The presence of various bioactive compounds justifies the use of the whole plant for various Aliment by traditional pracditioners.It could be concluded that *Barleria cristata* contains various bioactive compounds and signifies

Table 3

GC – MS High percentage compounds

A Selected and identified compound in the highest (GC-MS) m/z percentage area of *Barleria cristata* in Ethyl acetate, chloroform and ethanol leaves extract.

Structure and naming of the compound	Name of Extract	Molecular Formula	MW	Retention Time Values	m/z Area%	Biological Activity	PubChem Reference CID: No
 6-Amino-1,2,3,4tetrahydroindan-5,7-Dione, N- Carbethoxy	Ethyl acetate	$C_{12}H_{15}NO_4$	237.25	17.22	65	Neurotransmitter Agent	567162
 9, 12, 15 – Octadecatrienoicacid,methyl ester,[ZZZ]	Ethyl acetate	$C_{19}H_{32}O_2$	292.46	17.73	100	Neurogenerative Diseases	5319706
 1,2-Benzenedicarboxylic acid, butyl 2-methyl propyl ester	Chloroform	$C_{16}H_{22}O_4$	278.34	17.68	100	Plasticizers	3026
 p-Decylphenoxy acetic acid	Chloroform	$C_{18}H_{28}O_3$	292.41	18.48	76	Plant growth regulation, Antibacterial activity	5280411
 2,6,6-Trimethyl-2-3-[3-oxo -3 – phenylpropenyl) cyclohexane	Ethanol	$C_{18}H_{22}O_2$	270.37	17.28	95	Vasomotor symptoms, atrophic vaginitis, deficiency.	5870

Molecular dockings

The chloroform leaves extract compound of P-Decylphenoxy acetic acid possess good results of hydrogen bond interaction with ASP66 residues found in the identified pocket (Fig 1 and Fig 2). Two Carbon – hydrogen Bond and one conventional hydrogen bond respectively i) C18 – H48… O [ASP66] distance 2.88, (C18 – H) carbon – hydrogen bond C18 atom was a sp^3 Hybridized carbon, which combined with sp^2 Hybridized oxygen were observed C-H… O bond interacted with protein residues ASP66. ii) [ASP66] C-H… 021 distance 2.59, here C-H carbon – hydrogen bond C was a sp^3 hybridized carbon, which combined with sp^2 hybridized O21 oxygen was observed C – H… O bond interaction with protein residues ASP66. Iii) Finally, one conventional Hydrogen bond [ASP66] O-H… O21 distance 1.99, here O-H oxygen – hydrogen bond O was a sp^3 hybridized oxygen which combined with sp^2 hybridized O21 oxygen. C-Docker, C- Docker interaction energy and libdock score also gave a promising result for these compounds. Besides, molecular docking study indicated the p-Decylphenoxy acetic acid could closely occupy the active site of target HLA – DR4 (PDB Id code 1D5X protein), inhibiting its activity, which had been identified to be a possible target site of the compounds.

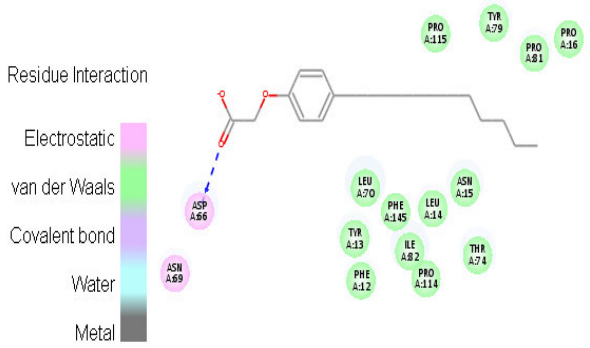


Figure 1
2D ligand – protein residues interaction scheme for chloroform leaves extract compound of p-Decylphenoxy acetic acid.

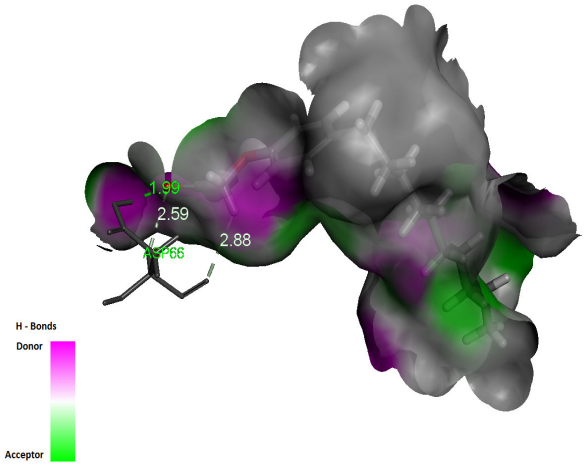


Figure 2
The docking pose of chloroform leaves extract compound of p-Decylphenoxy Acetic acid in the receptor site.

ADMET (absorption, distribution, metabolism, excretion, and toxicity) Studies

The ADMET plot is a 2D chart of ADMET_PSA_2D versus ADMET_AlogP98. The two sets of ellipses are for the prediction confidence space (95% and 99%) for the Blood Brain Barrier Penetration and Human Intestinal Absorption models, respectively. The graphical representation, image of these five compounds was shown in (Fig 3). Ethyl acetate, chloroform and ethanol plant extract of *Barleria cristata* leaves compound ADMET results were shown in (Table 4). Here, the compounds 1, 3, 4 and 5 shows good absorption ability (> 90% absorbed), which was shown inside the ellipse in (Fig 3). The compound (2) Ethyl acetate leaves extract of 10-Octadecenoic acid, methyl ester present in pink color Ellipse posses 99% absorption ellipse. The complete ADMET result of these five compounds was given in (Table 4).

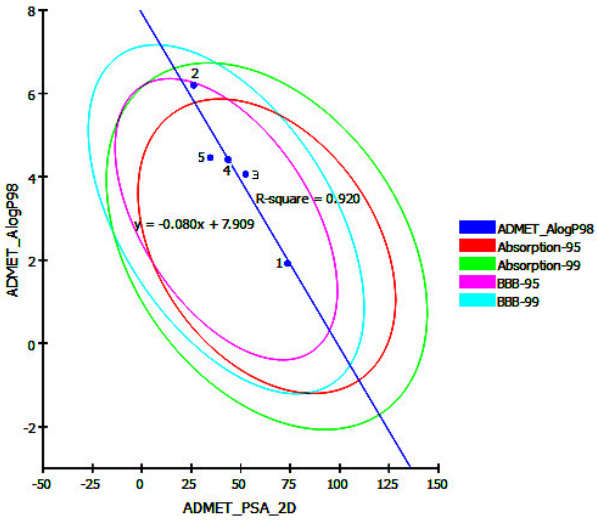


Figure 3
Graphical representation of ADMET properties of five compounds.

Table 4
ADMET (absorption, distribution, metabolism, excretion, and toxicity).

Ligand Name	Name of Extract	Solubility level	BBB Level	CYP2D6	Hepatotoxic	Absorption level	PPB	AlogP98	PSA_2D
6-Amino-1,2,3,4tetrahydroindan-5,7-Dione, N- Carbethox	Ethyl acetate	3	3	-1.71792	-4.13386	0	-4.2177	1.917	73.642
10-Octadecenoic acid, methyl ester	Ethyl acetate	2	0	-0.11469	-26.9205	1	-0.13158	6.197	26.23
1,2-Benzenedicarboxylic acid, butyl 2-methyl propyl ester	Chloroform	2	1	-0.79181	-8.66304	0	0.994718	4.061	52.461
p-Decylphenoxy acetic acid	Chloroform	2	1	-0.37917	-9.21271	0	3.45253	4.413	43.531
2,6,6-Trimethyl-2-3-[3-Oxo -3 –phenylpropenyl) cyclohexane	Ethanol	2	1	-0.38752	-5.81685	0	0.130927	4.458	34.601

DFT (Density Function Theory) Studies

The plot of the frontier orbitals HOMO-LUMO and Electrostatic potential was shown in (Fig 4). The isosurface of the electron density colored by the electrostatic potential molecules. By default, the isovalue of the electron density was 0.03, and the coloring scheme was spectrum Rainbow1 with a range from the default value -0.05 to 0.1 was shown in color (Fig 5). DFT Computed of chloroform leaves extracts compound of p-Decylphenoxy acetic acid was shown in (Table 5 and Table 6). According to the results was taken in $Dmol^3$ calculated text file in DS 4.0. The chloroform leaves extract of compound p-Decylphenoxy acetic acid contains total 243 number of valency orbitals, molecular charge is -1.0, active electron number was 160.0 and Electron temperature was found 0.005_Ha. The positive phase of the molecular orbital uses an isovalue of 0.01 and is colored blue, while the negative phase uses an isovalue of -0.01 and is colored red. It is the evident from the figure that, the HOMO levels are spread mainly over from acid group to LUMO phenyl group. The calculated energy value of HOMO and LUMO are 0.02706936 and 0.07383099 (Kcal/mol) and the frontier orbital gap is 0.04671 for chloroform leaves extracts of compound p-Decylphenoxy acetic acid. The lower value in the HOMO and LUMO energy gap makes clear the difference an interaction taking place within the molecules. It also explains the ultimate charge transfer interactions happen within the molecule, that influences its chemical and biological activities.

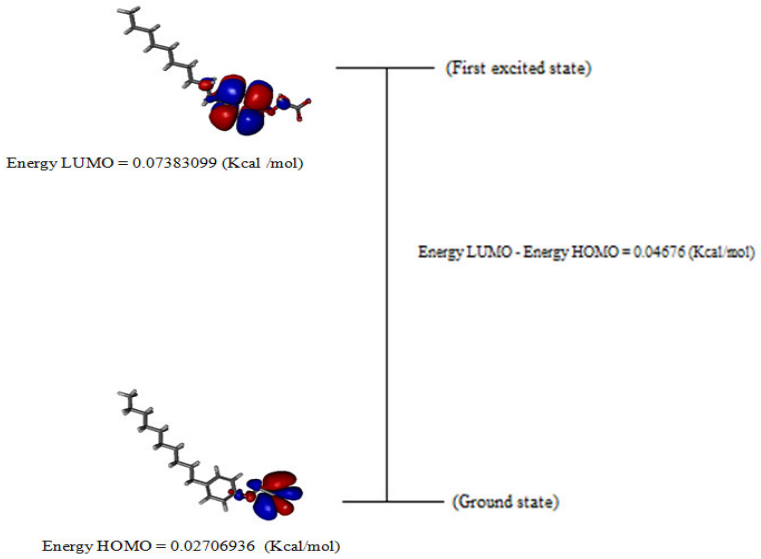


Figure 4
The molecular orbital and energy level for the HOMO and LUMO chloroform leaves extract of compound p-Decylphenoxy acetic acid.

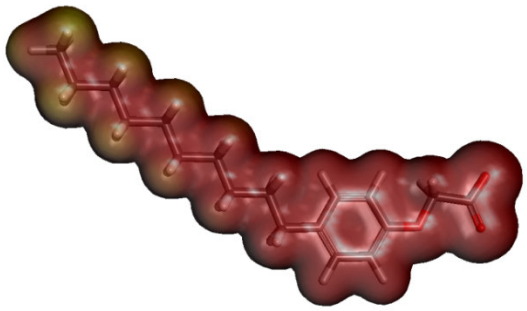


Figure 5
Molecular electrostatic potential map calculated at Dmol^3 properties of PWC function.

Table 5
DFT** Results **of p-Decylphenoxy acetic acid.

Name	Name of extract	Total Energy (Kcal/mol)	Binding Energy (Kcal/mol)	HOMO Energy (Kcal / mol)	LUMO Energy (Kcal/mol)
p-Decylphenoxy acetic acid	Chloroform	-919.72571272	-8.98702414	0.02706936	0.07383099

Table 6
DFT Results of p-Decylphenoxy acetic acid.

Name	Name of extract	Dipole Mag	Band Gap Energy (Kcal/mol)	Dipole X	Dipole Y	Dipole Z
p-Decylphenoxy acetic acid	Chloroform	17.50347282	0.04676163	11.18933259	13.459955	-2.88894

DISCUSSION

The phytochemical determination of biologically active compound of the plant is highly on the type of solvent used in the extraction procedure. Properties of a good solvent used in plant extraction include low toxicity, easy to evaporate at low heat, promotion of rapid physiologic absorption of the extract, preservation action, and inability to cause extract to complex or disassociate.[12, 13, 14] As the end product in extraction will contain traces of residual solvent, the solvent should be nontoxic and should not interfere with bioassay.[12, 13, 15] The plant is rich in diterpenes, cardiac glycosides, xanthoproteins. *Barleria cristata* leaf extract has alkaloids, cardiac glycosides, protein, flavonoids and similar results were reported earlier.[16] Ethanol has unipolar character to release polyphenols cells. It has high polarity which helps to detect the active component extraction of flavonoids, terpenoids, alkaloids.[12] Chloroform is used as an active compound of terpenoids and flavonoids.[12] The leaf extract of *Barleria cristata* possesses almost all the phytochemical that are tested here when compared to ethyl acetate extract. Ethanol extract is more abundant in saponins, flavonoids, diterpenes, cardiac glycosides, xanthoproteins. The Molecular Docking studies chloroform leaves extract compound of P-Decylphenoxy acetic acid showed higher ligand – receptor interaction and binding energy. The HLA-DR4 complexed with the dipeptide mimetic target to 1D5X protein was selected from a PDB data bank. The selected active site name AC1 was Presented in A chain residues viz. GLN9, GLU11, PHE32, SER53, PHE54, ASN62, VAL65, GLU172. Hydrogen bonding plays a vital role in the structure and function of biologically active molecules, the ligand – receptor interactions were scrutinized on the basis of hydrogen bonding. The outcome suggests that the chloroform leaves extract compound of p-Decylphenoxy acetic acid could closely occupy the active location of the HLA – DR4 target of 1D5X protein with binding higher energy – 77.19, compared to -47.309 of the same chloroform leaves extract of different compound viz., 1,2-Benzenedicarboxylic acid, butyl 2-methyl propylester. ADMET studies give insight into the pharmacokinetics property of the ligand compounds and properties of the molecules were predicted via Discovery Studio 4.0 (Accelrys). The studies of aqueous solubility, blood brain barrier level, CYP2D6, Hepatotoxicity and plasma protein binding were used to quantitatively predict ADMET characteristics of the chemical structure of the molecules.[23] Toxicity profiling of all the five ligand was performed by employing. Toxicity profile includes screening for aerobic biodegradability, developmental toxicity potentials, AMES mutagenicity, carcinogenicity and ocular & skin irritancy.[24] The pharmacokinetic study of the five compounds was predicted by DS 4.0. ADMET prediction was used to screen for sorting out those compounds represented in (Table 4), which followed the Lipinski's rule of 5. Oral administration is the most common route of drug administration. Most drugs in the marketplace are administered via the oral and it is one of the convenient Aqueous solubility used to predict the solubility of compounds in water at 25°C and it has eight different levels from 0-6. Aqueous solubility levels ranging from 0-2 indicates low solubility, level 3 indicates good solubility, level 4 indicates optimal solubility and level 5 indicates high solubility.[25] Therefore phytocompounds, ethyl acetate leaves extract of 6-Amino-1,2,3,4tetrahydroindan-5,7-Dione, N-

Carbethox showed good solubility range compared with other compound. Hence, these compounds can be administered as an oral drug. The blood-brain barrier (BBB) is a complex cellular system helps to maintain the homeostasis of the central nervous system (CNS) by separating the brain from the systemic blood circulation. Drugs acting at central nervous system should have the capacity to cross these barriers whereas other drugs crossing these barriers will cause unwanted side effects. The level 0&1 shows high penetration, level 2 shows medium penetration, level 3 shows low penetration and level 4 shows the undefined penetration level. Drugs have the possibility to cross the blood brain barrier. Ethyl acetate leaves extract compound of 6-Amino-1,2,3,4tetrahydroindan-5,7-Dione, N- Carbethox has low penetration level. The other compounds like ethyl acetate leaves extract compound of a 10-Octadecenoic acid, methyl ester, chloroform leaves extract of 1,2-Benzenedicarboxylic acid, butyl 2-methyl propyl ester, p-Decylphenoxy acetic acid and 2,6,6-Trimethyl-2-3-[3-Oxo -3 –phenylpropenyl) cyclohexane has high penetration level to cross the blood–brain barrier. Cytochrome 450 is the enzymes that catalyze the oxidation of organic substance. These are the major enzymes involved in drug metabolism. Most of the drugs undergo metabolism via the cytochrome P450 (CYP) enzymes. Cytochrome 450 (CYP450) predicts CYP2D6 enzyme inhibition using 2D chemical structure and it has 2 levels, namely 0 for non-inhibitor and 1 for inhibitor. 26 CYPs often have distinct roles in xenobiotic metabolism with active sites that enable broad and overlapping substrate specificity. All the ADMET resulted five compounds, respectively ethyl acetate, chloroform and ethanol phytocompounds has fallen in level 0 and these phytocompounds were non-inhibitor and unfavorable to inhibit CYP2D6 enzyme. Drugs continue to be pulled from the market with disturbing regularity because of the late discovery of hepatotoxicity. The liver synthesizes, concentrates and secretes bile acids and excretes other toxicants, such as bilirubin. Drug-induced injury to hepatocytes and bile duct cells can lead to cholestasis, in turn, causes intrahepatic accumulation of toxic bile acids and excretion products, which promotes further hepatic injury. Hepatotoxicity predicts potential organ toxicity for a wide range of structurally diverse compounds and it has 2 levels, namely 0 for non-toxic and 1 for toxic. All these five compounds are identified as the non - toxic level of favorable to cause dose-dependent liver injuries. Plasma binding protein helps to identify the binding of the inhibitors to the carrier protein in the blood. It is generally assumed that the only free drug can cross the membrane and bind to the intended molecular target therefore it is important to find the plasma protein binding. The drug can cross membranes and bind to the intended molecular target, and it is therefore important to estimate the fraction of drug bound to plasma proteins. Plasma protein has three levels of binding capacity namely level 0 has <90%, level 1 has >=90% and level 2 has >=95%. The phytocompound chloroform leaves extract compound of p-Decylphenoxy acetic acid has a binding capacity of >=95% to cross the membrane and bound to plasma protein. While the other phytocompounds ethyl acetate leaves extract compound of 6-Amino-1,2,3,4tetrahydroindan-5,7-Dione, N-Carbethox, 10-Octadecenoic acid, methyl ester, chloroform leaves extract compound of 1,2-Benzenedicarboxylic acid, butyl 2-methyl propyl ester and ethanol leaves extract compound of 2,6,6-Trimethyl-2-3-[3-Oxo -3 –phenylpropenyl)cyclohexane have binding capacity of <90% to cross the membrane and bound to the plasma protein. The DFT Calculations of HOMO–LUMO orbitals are also called frontier orbitals as they fall on the furthest boundaries of electron of the molecules. Both HOMO and LUMO are the most important orbital occur in chemical reactions. The frontier electron density makes clear numerous types of reactions in coupled systems and also for predicting the mass reactions in coupled system and also for predicting the greater part reactive site in π electron system. The HOMO energy depicts the capacity of electron donating. LUMO describes the capacity of electron accepting and the gap between HOMO and LUMO describes the molecular stability. The energy gap between HOMO and LUMO determine the stability of structures. The energy gap is basically responsible for the chemical and spectroscopic properties of the molecules. The conjugated molecules are depicted by a small HOMO – LUMO separation which is a consequence of a considerable amount of intra molecular charge transfer from electron donor group to the capable electron acceptor group through π – conjugated path. The DFT input, computed electronic parameter of charge was -1, function (PWE), Aux_density was octupole, cutoff_Global was 3 angstrom, self consistent field (SCF) density convergence was 1.0000e-004, self consistent field (SCF) charge mixing was 0.2000, self consistent field (SCF) interaction 50 was selected and Calculated properties of Mulliken, hirshfeld, electrostatic potential (ESP) fit, electrostatic moment was on. The HOMO and LUMO computed result was also carried out by D.S.4.0 $Dmol^3$ properties of PWCfunction.Calculations at DFT level, using the DMOL3 code.[26,27], were performed using: 1) the Local Density Approximation (LDA) for the exchange-correlation functional with PWC parameterization, and 2) the Generalized Gradient Approximation (GGA) with PBE parameterization. The DNP numerical basis set was adopted to expand the Kohn-Sham orbital for all electrons. The orbital cutoff, which is a parameter used to control the quality of the numerical basis set and the numerical integrations performed during the computations was set to a valid fine value of DNP basis set with SCF density convergence of 1.0e-6. This cutoff serves to reduce computation time with little impact on the accuracy of the results and is a very fine value for the atoms involved in the system under study. The total energy variation, which specifies the self-consistent field (SCF) convergence threshold, was selected to be 10-6 Ha, ensuring a well converged electronic structure for the system. The essential feature of Density Functional Theory (DFT), where the total energy of a multielectronic system is expressed as a function of the electron charge density, which is founded after solving the Kohn-Sham equations.[28,29] Another important approach to work through quantum mechanics calculations on large molecules or molecular systems is the use of fragmentation methods to make it more computationally acceptable and, at the same time, maintain the better accuracy of the quantum calculation.[30]

CONCLUSION

Solvent extraction of *Barleria cristata* leaf was rich in various medicinally important classes of compounds like alkaloids, saponins, flavonoids, terpinoids and xanthoproteines were inspected by qualitative photochemical analysis manner. The chloroform leaves extract of compound p-Decylphenoxy acetic acid was a promising compound and safe as confirmed by *in silico* molecular docking and ADMET in DS.4.0. Using the target HLA – DR4, responsible for rheumatoid arthritis. Thus the compound can be further extended for *in vitro* and *in vivo* analysis for the treatment of rheumatoid arthritis.

ACKNOWLEDGEMENTS

The Bioinformatics infrastructure Facility Centre of the Department of Biotechnology (DBT). inQueen Mary's college, Chennai – 600004.

CONFLICT OF INTEREST

Conflict of interest declared none.

REFERANCES

1. Balkwill MJ, Aalkwill K. A preliminary analysis of distribution pattern in a large,pantropical genus, *Barleria* L. (Acanthaceae). *J. Biogeography* 1998;25:95 - 110.
2. Mabberley DJ. Mabberley's Plant-Book. A portable dictionary of plants, their classification and uses. Third Edition, Cambridge University Press, Cambridge. 2008;7-18:1-1021.
3. Wagner WL, Herbst DR and Shomer S H. Manual of the flowering plants of Hawaii. Revised edition, Bernice. Bishop Museum Special Publications: Bishop Museum Press. Honolulu. 1999;1919-20.
4. Gambhire M, Juvekar A and Wankhede S. Evaluation of anti-inflammatory activity of methanolextractof *Barleria cristata* leaves by *in vivo* and *in vitro* methods.*Internet J. Pharmacol.* 2008; 7:1-4.
5. Charoenchai P, Vajrodaya S, Somprasong W, Mahidol C, Ruchirawat S. And Kittakoop P. Part 1: Antiplasmodial, cytotoxic, radical scavenging and antioxidant activities of Thai plants in the family Acanthaceae. *Planta. Med.* 2010;76: 1940-3.
6. Hemalatha K, Hareeka N and Sunitha D Chemical constituents isolated from leaves of *Barleria cristata* Linn. *Int. J. Pharma and Biosci.* 2012:3(1); 609-615.3.
7. Abd El-Mawla AMA, Ahmed AS, Ibraheim ZZ. And Ernst, L. Phenylethanoid glycosides from *Barleria cristata* L. callus cultures. *Bull. Pharm. Sci.* 2005;28:199-204.
8. Sriram S, Sasikumar CG. Pharmacognostic Standardization and Physicochemical analysis of the leaves of Barleria montana Wight & Nees. Asian Pac J Trop Biomed., 2012. 1: 1-3.
9. KR Kirtikar and BD. Basu, "Indian Medicinal Plants", Jayyed Press. 1975; Delhi-6, Vol. III, 2nd Ed., p. 1876.
10. AS Ahmed M.Sc. Thesis, Faculty of Pharmacy, Assiut University, Assiut, Egypt (1990).
11. NA El-Emary MA. Makboul M A. Abdel-Hafiz and AS. Ahmed, Bull. Pharm. Sci., Assiut University, 1990;13, 65.
12. Pranshant Tiwari, Bimlesh Kumar, Mandeep Kaur, Gurpreet Kaur, Harleen Kaur. Phytochemical screening and Extraction : A Review. International Pharmaceutica Sciencia. 2011;1(1): 99- 106.
13. Das K, Tiwari RKS, Shrivastava DK. Techniques for evaluation of medicinal plant products as antimicrobial agent: Current methods and future trends . Journal of Medicinal Plants Research. 2010;4(2): 104-111.
14. Hughes I. Isoprenoid compounds and phenolic plant constituents, Elsevier, New York, N.Y.Sci.Afr.Mag. 2002;9(56), 502.
15. Ncube N. Afolayan SAJ, Okoh Al. Assessment techniques of antimicrobial properties of natural compounds of plant origin: current methods and future trends. Afr. J. Biotechnol. 2008;7(12):1797-1806.
16. Rajasekaran Narmadha, Kanakasabapahi Devaki.Barleriya cristata linn.: Phytochemical screening and HPDLC analysis. International research journal of pharmacy. 2012,3(7).
17. Zakia Khanam, Chew Shwu Wen , Irshad Ul Haq Bhat. Phytochemical screening and antimicrobial activity of root and stem extracts of wild Eurycoma longifolia Jack (Tongkat Ali) . Journal of King Saud University – Science .; 2014;1-8.
18. Solomon Charles Ugochukwu , Arukwe Uche I. And Onuoha Ifeanyi. Preliminary phytochemical screening of different solvent extracts of stem bark and roots of Dennetia tripetala G. Baker . Asian J. Plant Sci. Res., 2013;3(3):10-13.
19. Chandra Shekar Misra, Kumar Pratyush, Lipin Dev MS, Joel James, Arun Kumar Thaliyil Veeettil and Thankamani VA. comparative study on phytochemical screening and antibacterial activity of roots of Alstonia scholaris with the roots , leaves and stem bark. Int. J. Res. Phytochem. Pharmacol., 2011;1(2):77-82.
20. Ashvin Godghate , Rajaram Sawant and Ashok Sutar.Phytochemical analysis of ethanolic extract of roots of carrisa carandus linn.rasayan J.Chem. 2012; 5(4): 456-459.
21. R Suman Kumar, CVenkateshwar, G Samuel, S Gangadhar, Phytochemical Screening of some compounds from plant leaf extracts of Holoptelea integrifolia (Planch.) and Celestrus emarginata (Grah.) used by Gondu tribes at Adilabad District, Andrapradesh, India.International Journal of Engineering Science Invention. 2013;2(2):65-70.
22. De-Fatima A, Modolo LV, Conegero LS, Pilli RA, Ferreira CV, Kohn LK, de-Carvalho JE. Lactones and their derivatives: biological activities,

mechanisms of action and potential leads for drug design. Curr. Med. Chem. 2006;13:3371-84.
23. Daisy P and Suveena S, Solutions to pharmaceutical issues for anti-cancer drugs by accord excel, Asian journal of pharmaceutical and clinical research. 2012;5:149-158.
24. Xia X, Maliski EG, Gallant P, Rogers D. Classification of kinase inhibitors using a Bayesian model. J Med Chem. 2004; 47(18): 4463-70.
25. Bevan CD and Lloyd RS. A high-throughput screening method for the determination of aqueous drug solubility using laser nephelometry in Microtiter plates. 2000; Analytical Chemistry 72: 1781–7.
26. Delley B. An all-electron numerical method for solving the local density functional for polyatomic molecules. J Chem Phys. 1990;92: 508-17.
27. Delley B. From molecules to solids with the DMol[sup 3] approach. J Chem Phys. 2000;113: 7756-64.
28. Hohenberg P, Kohn W. Inhomogeneous Electron Gas. Phys Rev. 1964;136: 864- 71.
29. Kohn W, Sham LJ Self-Consistent Equations Including Exchange and Correlation Effects. Phys Rev. 1965;140: 1133–38.
30. Gordon MS, Fedorov DG, Pruitt SR, Slipchenko LV. Fragmentation Methods: A Route to Accurate Calculations on Large Systems. Chem Rev. 2012;112: 632-672.

Reviewers of this article

Priya Roosevelt M.Sc, M.Sc, Ph.D

Post Doctoral Fellow , Dept of chemistry ,
College of chemistry , Jilin University ,
2699 Qianjin Street , Changchun 130012 ,
Peoples Republic of China .

Prof. Y. Prapurna Chandra Rao

Assistant Proffessor, KLE University,
Belgaum, Karnataka

Prof.Dr.K.Suriaprabha

Asst. Editor , International Journal
of Pharma and Bio sciences.

Prof.P.Muthuprasanna

Managing Editor , International
Journal of Pharma and Bio sciences.

We sincerely thank the above reviewers for peer reviewing the manuscript