ISSN: 0974-2115
www.jchps.com Journal of Chemical and Pharmaceutical Sciences

In silico anticancer analysis of bioactive compounds in *Vitex altissima l* and *Vitex leucoxylon l*

Santhanabharathi Naganathan[1], Vivek Pazhamalai*[2], Anupama Natarajan[1], Hemachandran Munusami[2], Gayathri Kothandaraman[1]

[1]Dept. of Biotechnology, Vel Tech High Tech Dr.RangarajanDr.Sakunthala Engineering College, Avadi, Chennai.
[2]Dept. of Biotechnology, Sree Sastha Institute of Engineering and Technology, Chembarambakkam, Chennai.

***Corresponding Author: E-Mail: vivek.biotech@sasthaenggcollege.com**

ABSTRACT

The knowledge of the traditional plants in India is a collection over millennia by our ancient people. The scientific analysis is carried out all over in India since Vedic times (i.e. greater than 6000 BC), and are present in a group of herbal preparations of the Indian traditional health care system (Ayurveda) proposed for their valuable anti-cancer and other valuable properties. *Vitex*species were used in Ayurveda for its anti- cancer activity for several years. However, the present study deals with the two important on coproteins because of its complex behavior and dangerous effects. The current study deals with the comparative analysis of the traditional plant against two majorcancer causing proteins. The plants which were subjected to phyto chemical analysis showed that 22 bioactive compounds are present in *Vitexaltissima* L and 18 compounds are present in *Vitexleucoxylon* L. These compounds were docked with the on coproteins to know the best binding site using various bio informatics software and tools. The interaction rate was determined by using molecular docking software between herbal compounds against on coproteins based on binding free energy requirements. The results were analyzed and it showed that most of the herbal compounds were effective against the target proteins. Hence, this study will form the basis for designing therapeutic lead molecules from the traditional plants which could results into massive reduction in experimental validations and clinical trials. Also, it reduces the disease at much faster rate, as well as that it avoids the harmful side effects.

KEY WORDS: Ayurveda, *Vitexaltissima* L, *Vitexlevcoxylon* L, oncoproteins, Molecular docking software, Clinical trials, Therapeutic lead molecules.

1. INTRODUCTION

Cancer is considered to be one of the most severe health issues in the modern era. Cancer, medically termed as a "Malignant neoplasm"is a comprehensive group of several diseases, comprising primarily of uncontrolled level of cell division. In this case, thecell cycle continues without any hindrance, leading to the formation of solid mass of cells 'Tumor'. Cancer is a typical disease which needs intense monitoring and frequent observation. The initial stage of tumor is generally termed as the primary tumor and later, it gets transformed into huge cell mass. These cell mass spread to the other sites of the body, thereby blocking the vessels or organs; the process is termed as 'Metastasis'. There are over 200 different known cancers that afflict humans. Conventional treatment is normally directed on cancer diseases by chemotherapeutic agent that can effectively remove the totality of tumor; however, this treatment can cause many side effects. Therefore, the search of the effective organic compound from plants with the target on genes regulating the cell growth or cell proliferation with minimum side effects is very much essential. Nowadays, there are a lot of researches and developments of bioactive compounds from medicinal plants which is highly potential and selective anticancer activity, especially to inhibit the activity of cancerous cells. At present, several siddha doctors work in collaboration with oncologists during chemotherapy and radiotherapy. A conventional chemotherapy destroys the WBC and platelets along with the cancer cells, thereby damaging their immune system. It was also evident that the cancer patients taking siddha medicines, during chemotherapy and radiation therapy, showed lesser side effects, i.e: their WBC and platelet counts don't fall drastically after therapy. The foremost objective of this research paper is to identify the active compound from the natural plants that have been used by the southern part of India for the treatment of several types of cancer. The target site of the cancer was identified and was made to interact with the active phytochemical compounds using insilico approaches.

1.1. *Vitex* sp.: Various plants are found to possess medicinal values and are in use for the treatment of various chronic and acute diseases. The genus *Vitex*comprises of over 270 species of various trees and shrubs and is mainly found in the tropical and subtropical regions, while some species are widely spread in the temperate zones. *Vitexaltissima* L. is a big tree with a grey, scaly, fibrous bark. The leaves are 3-foliolate; petiole angular or winged; the leaflets are sub sessile, while the flowers are bluish-white. It is commonly known as 'Mayilainotchi' (in Tamil) and is broadly distributed in Southern parts of East Asia. It is widely used for curing stomatitis, cardiac diseases, anorexia, leprosy, worm infestation, rheumatic swellings and chest pains. It also has anti-inflammatory and antioxidant activities. Stem bark is used for the treatment of ephemeral fever, snake bite. Leaves are crushed and is applied over the wounds, and they are also used against skin allergies, snake and scorpion bites and rheumatism.

Vitex leucoxylon L., commonly known as "nir-notchi" (in Tamil), is another important medicinal plant extensively found in the Eastern Ghats and Deccan plateau regions located in India. The leaves of *V.leucoxylon* L are used in traditional medicine (mainly in SSM) for relieving headache, fever. General pharmacological studies revealed various properties of various aqueous and ethanolic extracts of *V. Leucoxylon* L (mainly their leaves) such as analgesic, anti- inflammatory, anti-parkinsonianand anti-microbial activities. Sarma (1990) used the alcoholic leaf extract of these plants for understanding the anti-inflammatory and wound healing properties in acute inflammation model. On analyzing the roots and bark, they are found to be used as astringent and the roots are reported to be used as a febrifuge.

1.2. Cancer targets:

1.2.1. BCL2: BCL- 2 (B-cell lymphoma 2) is one of the regulator proteins which regulate the apoptosis. It suppresses the apoptosis of cell by preventing the activation of caspases. BCL- 2 is generally a proto- oncogene positioned in the chromosome 18. This gene produces the integral membrane protein of 29kDa. Mutation in this protein leads to anti-apoptosis activity; consequently, the cell will undergo uncontrolled growth. The gene due to various changes undergoes translocation from chromosome 18 to 14 position (immunoglobulin heavy chain locus), leading to follicular lymphoma. Correspondingly, various impairment or mutation to BCL-2 protein has also been identified as a cause of number of cancers, including melanoma, breast cancer, prostate cancer, chronic lymphocytic leukemia, and lung cancer.

1.2.2. HER2: Receptor tyrosine-protein kinase erbB-2 or HER2 (Human epidermal growth factor receptor 2) is one of the members of the epidermal growth factor receptor (EGFR/ERBB) family. This receptor is located on the surface of all breast cells and is responsible for the growth of breast cells. Increased amounts of HER 2 lead to the uncontrolled growth of the breast cells. The overexpression of HER 2 oncogene has been revealed to play an important role in the development and progression of definite aggressive types of breast cancer. In recent years, many patients of breast cancer were found to have increased levels of HER 2 protein in their urine and blood samples.

2. MATERIALS AND METHODS

2.1. Selection and Retrieval of protein structure from database: In this study, two cancer proteins namely BCL-2 (PDB ID: 2O22) and HER2 (PDB ID: 4HRL) were chosen. The correct crystal structure for these cancer targets has been obtained from RCSB Protein Data Bank (http://www.pdb.org).

2.2. Construction or retrieval of Ligand structure: The compounds reported in Fourier transform infrared spectroscopy (FTIR) analysis of *Vitexaltissima* L and *Vitexleucoxylon*L wereused in this present study. Chem Sketch is a chemically advanced quick drawing interface freeware, designed and developed by Advanced Chemistry Development, Inc., (http://www.acdlabs.com). It was used to design the structure of most of the ligands. Using the "draw mode" of Chem Sketch, the three dimensional optimizations and the essential ligands were generated and it was then saved in '**.**mol' format. The remaining phytochemical structures were retrieved from Pub Chem compound database.

2.3. Preparation of protein targets: The water molecules present will disturb the binding nature of the compounds to the active site thereby reducing the efficiency of the compound against the target proteins. Thus, by using Argus lab, crystallographic water molecules and other unwanted ligands were cleaved and were removed from the protein. Crystallographic disorders and void atomic spaces were corrected to improve binding energy. Then, the protein was subjected to energy minimization and on the final stage, addition of hydrogen atoms to the target protein molecule before docking was performed.

2.4. Ligand preparation and identification: Geometrical optimizations of the ligands were performed based on the Hartree-Fock (HF) calculation method using Argus Lab 4.0.1 software.

2.5. Binding site detection: Meta Pocket is one of the tools used for the estimation of binding site. It uses the interaction energy between the protein and a simple Vander Waals probe to locate energetically favorable binding sites. Here, Meta Pocket server (http://projects.biotec.tu-dresden.de/metapocket/index.php/) was analyzed further for the identification of the most potential active site for binding and interaction of the target protein and the ligand.

2.6. Molecular Docking using Argus Lab 4.0.1: In silico modeling is a modern approach for faster and quicker analysis of efficient binding. It finds a way for the traditional drug testing compounds that reduces the time consuming multi step process against biological screens. Molecular docking is a method to confirm and locate the binding mode and interaction energy for the ligands with the target protein without any complex wet lab analysis and screening.

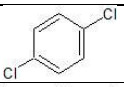
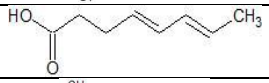
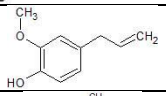
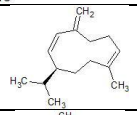
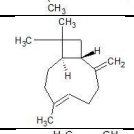
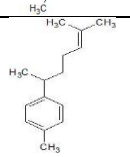
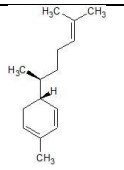
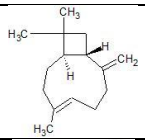
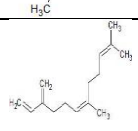
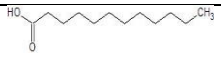
Molecular docking was performed in Argus lab. Upon finding out the predicted binding residues, the grid box was built and the molecular docking was performed. All the computational docking studies were performed using Argus lab 4.0.1, installed in a single machine running on a 2.2 GHz core i3 processor with 6 GB RAM and 320 GB hard disk with windows 8 as an operating system. Argus Lab is a computerized structure program, generally based on the quantum mechanics. Argus Lab is used to predict the potential energies, molecular structures; geometrical optimization of structure, vibrational frequencies of various atom coordinates, bond length and reactions

pathway. Target proteins were docked against the 21 ligands (obtained from the PDB) using Argus Lab 4.0.1 (Mark A. Thompson, Planaria Software LLC, Seattle, WA, USA, (http://www.arguslab.com)) to find the reasonable binding geometries and explore protein- ligand interactions. The docking was mainly targeted only on to the predicted active site. Simulations for docking were performed by selecting "Argus Dock" as the docking engine. The residues (in the receptor) that have been selected were defined to be a part of the binding site. A 0.4 Å spacing was used between the grid points and an comprehensive search was performed by enabling "High precision" option in Docking precision menu, "Dock" was chosen as the calculation type, "flexible" for the ligand and the A Score was used as the scoring function. The A Score function was generally used to calculate the binding energies of the resulting docked structures. All the compounds present in the data file were docked into the active site of cancer targets, using the same protocol. After the completion of docking, the docked protein (protein-ligand complex) was analyzed to examine the type of interactions. The docking poses saved for each compound were ranked according to their dock score function. The pose having the highest dock score was selected for further analysis.

3. RESULTS

Proteins and ligands were retrieved and prepared for docking. Binding sites were predicted by online tool and were used during docking. Binding energy requirement between the cancer targets and 21 phyto-compounds by molecular docking was calculated and tabulated below in kcal/mol.

Table.1.FTIR analyzed result of leaf extracts of *Vitexa ltissima* L. and the results of docking with the target protein

Phytochemical	Molecular Formula	Structure	Cancer Targets (Pdb Id)	
			BCL2(2o22)	HER2 (4hrl)
1, 4-Dichloro Benzene	$C_6H_4Cl_2$		-10.01	-8.59
4, 6-Octadienoic Acid	$C_8H_{12}O_2$		-9.633	-8.71
Eugenol	$C_{10}H_{12}O_2$		-9.658	-9.13
Germacrene D	$C_{15}H_{24}$		-	-
Caryophyllene	$C_{15}H_{24}$		-14.89	-10.11
Benzene,1-(1,5-Dimethyl-4-Hexenyl)-4-Methyl,[S-(R*, S*)]	$C_{15}H_{24}$		-14.67	-11.52
1, 3-Cyclohexadiene, 5-(1, 5-Dimethyl-4-Hexenyl)-2-Methyl	$C_{15}H_{24}$		-15.21	-11.61
À-Caryophyllene	$C_{15}H_{24}$		-14.89	-10.11
1,6,10-Dodecatriene, 7, 11-Dimethyl-3-Methylene-[Z]	$C_{15}H_{24}$		-14.79	-11.89
Dodecanoic Acid	$C_{12}H_{24}O_2$		-11.464	-8.45

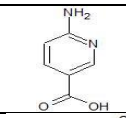
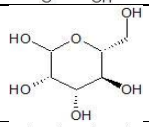
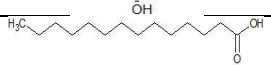
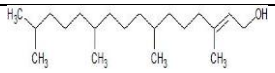
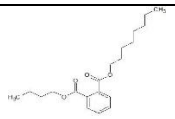
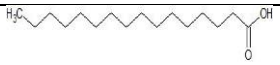
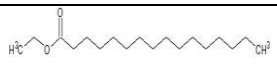
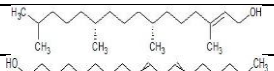
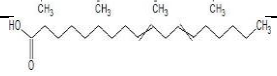
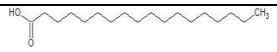
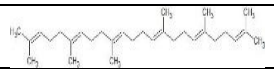
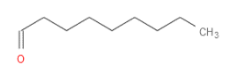
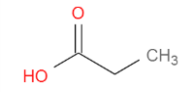
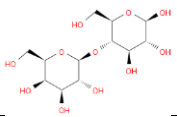
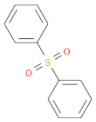
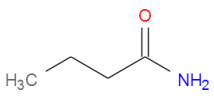
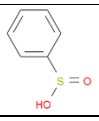
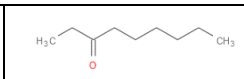
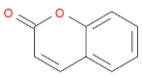
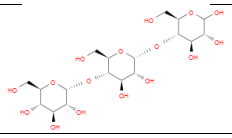
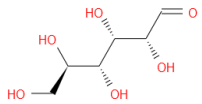
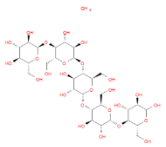
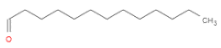
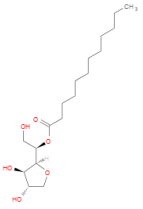
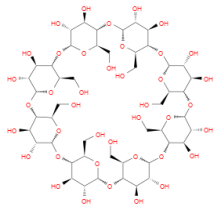
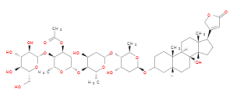
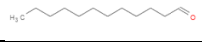
3-Pyridine Carboxylic Acid,6-Amino	$C_6H_6N_2O_2$		-7.26	-7.121
D-Mannose	$C_6H_{12}O_6$		-4.67	-4.70
Tetradecanoic Acid	$C_{14}H_{28}O_2$		-11.69	-7.995
3,7,11,15-Tetramethyl-2-Hexadecen-1-Ol	$C_{20}H_{40}O$		-17.31	-11.50
1, 2-Benzene Dicarboxylic Acid, Butyl Octyl Ester	$C_{20}H_{30}O_2$		-14.89	-10.55
N-Hexadecanoic Acid	$C_{16}H_{36}O_2$		-12.94	-8.45
Hexadecanoic Acid Ethyl Ester	$C_{18}H_{36}O_2$		-15.22	-10.54
Phytol	$C_{20}H_{40}O$		-17.30	-11.50
9, 12-Octadecadienoic Acid [Z, Z]	$C_{18}H_{36}O_2$		-9.88	-10.74
Octadecanoic Acid	$C_{18}H_{36}O_2$		-15.87	-10.54
Squalene	$C_{30}H_{50}$		-17.29	-11.80

Table.2. FTIR analyzed result of leaf extracts of *Vitex leucoxylon* L. and the results of docking with the target protein

Compound Name	Molecular Formula	Structure	Cancer Targets (PDB ID)	
			BCL2 (Kcal/Mol)	HER2 (Kcal/Mol)
Nonyl Aldehyde	$C_9H_{18}O$		-11.682	-4.431
Triacontane	$C_{30}H_{62}$		-11.394	-9.001
Propionic Acid	$C_3H_6O_2$		-12.469	-8.900
(+)-Beta-D-Lactose	$C_{12}H_{22}O_{11}$		-8.730	-7.776
Phenyl Sulfone	$C_{12}H_{10}O_2S$		-13.180	-10.019
Butyramide	C_4H_9NO		-9.902	-7.813
Benzenesulfinic Acid	$C_6H_6O_2S$		-7.738	-6.291

3-Nonanone	$C_9H_{18}O$		-9.903	-8.173
Coumarin	$C_9H_6O_2$		-10.998	-9.482
Maltotriose Hydrate	$C_{18}H_{32}O_{16}$		-11.688	-10.525
Dextrin	$C_6H_{12}O_6$		-6.203	-5.553
Maltopentaose Hydrate	$C_{30}H_{54}O_{27}$		-13.180	-10.353
Tridecanal	$C_{13}H_{26}O$		-7.200	-6.870
Sorbitanmonolaurate	$C_{18}H_{34}O_6$		-8.926	-7.952
Gamma-Cyclodextrin Hydrate	$C_{48}H_{80}O_{40}$		-9.991	-8.775
Lanatoside A	$C_{49}H_{76}O_{19}$		-7.525	-9.628
Dodecyl Aldehyde	$C_{12}H_{24}O$		-7.707	-6.335

DISCUSSION

The phytochemicals that were used in this study revealed a broad range of the binding energies (from (-4.67kcal/mol) to (-18.47 kcal/mol)) which was in very good agreement with the standard and ideal binding energy. 3, 7, 11, 15-Tetramethyl-2-hexadecen-1-ol showed the maximum effectiveness against BCL- 2. Similarly 1, 6, 10-Dodecatriene, 7, 11-dimethy l-3-methylene-[Z] showed the maximum effectiveness against HER2. These two compounds were found in *Vitex altissima* L. Thus, in comparison with the *Vitex leucoxylon* L., *Vitex altissima* L. alone can be effectively used for the cancer treatment without any adverse side effects. These compounds also showed maximum binding energy in comparison with that of the standard drugs. Correspondingly, the side effects of these compounds will be profoundly less because of its source (natural plant source).

4. CONCLUSION

The protein-ligand interaction generally has a foremost role in the structural based drug designing. In this present work, the potential drugs for treating Cancer were identified by selecting the suitable receptors for cancer targets. By applying computational approaches, it has been tried to understand the mechanism of interactions and binding affinity between phytochemicals and cancer targets. Hence these natural compounds could be effectively used as the template for designing the therapeutic lead molecules which could results into massive reductions in therapeutics development time. This study is just the beginning of various experimental validation and clinical trials to establish these phytochemicals as more potent drug for treating different cancers in general (mainly, breast cancer). In future, the ADME/T (Absorption, Distribution, Metabolism, Excretion / Toxicity) properties of these compounds

can also be calculated using the bioinformatics tools, thus reducing the time and cost in drug discovery process. These results will be regarded as a decisive and most significant factor for determining a lead phytochemical for future drug discovery and development process.

REFERENCES

Ab Mitri Z, Constantine T, O'Regan R, The HER2 Receptor in Breast Cancer: Pathophysiology, Clinical Use, and New Advances in Therapy, Chemother Res Pract., 2012, 743193.

Alasdair T.R Laurie and Richard M. Jackson, Q-sitefinder: an energy-based method for the prediction of protein–ligand binding sites, Bioinformatics, 21 (9), 2005, 1908–1916.

Ayyanar M, Ignacimuthu S, Herbal medicines for wound healing among tribal people in Southern India: Ethnobotanical and scientific evidences, International Journal of Applied Research in Natural Products, 2 (3), 2009, 29-42.

Ayyanar M, Ignacimuthu S, Medicinal plants used by the tribals of Tirunelveli hills, Tamil Nadu to treat poisonous bites and skin diseases, Indian J Trad Knowledge, 4 (3), 2005, 229-236.

Bruncko M.L, Oost T.K, Belli B.A, Ding H, Joseph M.K, Kunzer A, Martineau D, McClellan W.J, Mitten M, Ng S.C, Nimmer P.M, Oltersdorf T, Park C.M, Petros A.M, Shoemaker A.R, Song X, Wang X, Wendt M.D, Zhang H, Fesik S.W, Rosenberg S.H, Elmore S.W, Solution structure of the anti-apoptotic protein BCL-2 in complex with an acyl-sulfonamide-based ligand, J.Med.Chem., 50, 2007, 641-662.

Brylinski M and Skolnick J, A threading-based method (FINDSITE) for ligand-binding site prediction and functional annotation, PNAS, 105, 2008, 129-134.

Chanda Y.R, The wealth of India: A dictionary of Indian Raw materials and Industrial products, Publication and Information Directorate, CSIR, New Delhi, 1982, 520-521.

Cleary M.L, Smith S.D, Sklar J, Cloning and structural analysis of cDNAs for bcl-2 and a hybrid bcl-2/immunoglobulin transcript resulting from the t (14;18) translocation, Cell 47 (1), 1986, 19–28.

Imran Ali, Waseem A, Wani and Kishwar Saleem, Cancer Scenario in India with Future Perspectives, Cancer Therapy, 8, 2011, 56-70.

Jost C.L, Schilling J, Tamaskovic R, Schwill M, Honegger A, Plückthun A, Structural basis for eliciting a cytotoxic effect in HER2-overexpressing cancer cells via binding to the extracellular domain of HER2, Structure, 21, 2013, 1-13.

Lisina K.V, Eashwaranurugesh, Shanmughaveliramanayagam, In Silico Analysis of Compounds Characterized from *Nymphaeastellata* Flower with MUC1, Int. J. Pharm. Sci. Rev. Res., 23 (2), 2013.

Makwana H.G, Ravishankar B, Shukla V.J, Vijayan N.P, Sasikala C.K, Saraswathy V.N, and Bhatt S.V, General pharmacology of *Vitexleucoxylon* Linn leaves, Indian J. Physiol. Pharmacol., 38, 1994, 5-100.

Meena A.K, Niranjan U.S, Rao M.M, Padhi M.M, Babu R, A review of the important chemical constituents and medicinal uses of *Vitex* genus, Asian Journal of Traditional Medicines, 6 (2), 2011, 54-60.

Narayana Rao K, Thammanna T, Medicinal Plants of Tirumala, T.T.D, Tirupati, India, 1990.

Narayanan M.K.R, Mithunlal S, Sujanapal P, Anil Kumar N, Sivadasan M, Alfarhan A.H, Alatar A.A, Ethnobotanically important trees and their uses by Kattunaikka tribe in Wayanad Wildlife Sancturary, Kerala, India, Journal of Medicinal Plants Research, 5 (4), 2011, 604-612.

Padmalatha K, Jayaram K, Raju N.L, Prasad M.N.V, and Arora R, Ethnopharmacological and Biotechnological Significance of *Vitex*, Bioremediation, Biodiversity and Bioavailability, 3 (1), 2009, 6-14.

Parrotta J.A, Healing plants of Peninsular India, CABI Publishers, USA, 770.

Patel B, Das S, Prakash R and Yasir M, Natural bioactive compound with anticancer potential, International Journal of Advances in Pharmaceutical Sciences, 1, 2010, 32-41.

Pragada P.M, Rao G.M.N, Ethno veterinary medicinal practices in tribal regions of Andhra Pradesh, India, Bangl J Plant Taxon., 19 (1), 2012, 7-16.

Rajakumar N, Shivanna M.B, Traditional herbal medicinal knowledge in Sagar taluk of Shimoga district, Karnataka, India, Indian Journal of Natural Products and Resources, 1 (1), 2010, 102-108.

Rajeswari S, Krishnapriya S, Anticancer Activities of Phytochemicals from a Wood Rot Mushroom *Phellinusadamantinus* - In Silico and *In Vitro* Approach, International Conference on Bioscience, Biochemistry and Bioinformatics, 5, 2011.

Reddy K.N, Reddy C.S, Raju V.S, Ethnomedicinal observations among the Kondareddis of Khammam district, Andhra Pradesh, India, Ethnobotanical Leaflets, 12, 2008, 916-926.

Rohini K, Srikumar P.S, In Silico Approach of Anticancer Activity of Phytochemical Coumarins Against Cancer Target Jnks, International Journal Of Pharmacy And Pharmaceutical Sciences, 5 (3), 2013.

Saba Ferdous, Muhammad Usman Mirza, Usman Saeed, Docking Studies reveal Phytochemicals as the long searched anticancer drugs for Breast Cancer, International Journal of Computer Applications, 67 (25), 2013.

Sarma S.P, Aithal K.S, Srinivasan K.K, Udupa A.L, Kumar V, Kulkarni D.R, and Rajagopal P.K, Anti-inflammatory and wound healing activities of the crude alcoholic extract and flavonoids of *Vitexleucoxylon,* Fitoterapia, 61, 1990, 263-265.

Sathish S.S, Janakiraman N, Johnson M, Phyto chemical analysis of *Vitexalits sima* L. Using UV-Vis, FTIR and GC-MS, International journal of Pharmaceutical Science and Drug Research, 4 (1), 2012, 56-62.

Sridhar C, Rao K.V, Subburaju G.V, Flavonoids, triterpenoids and a lignin from *Vitexaltissima*, Phytochemistry, 66, 2005, 1707-1712.

Sridhar C, Subburaju G.V, Venkateshwaralu Y, Venugopal R.T, New acylatediridoid glycosides from *Vitexaltis sima*, Journal of Natural Products, 67, 2004, 2012-2016.

Thangaraj Sindhu, Sundaraj Rajamanikandan, Dhanapal Durgapriya, Jebamalai Raj Anitha, Selvaraj Akila and Velliyur Kanniyapan Gopalakrishnan, Molecular docking and qsar studies on plant derived bioactive compounds as Potent inhibitors of dekoncoprotein, Asian Journal of Pharmaceutical and Clinical Research, 4 (2), 2011.