ELUCIDATION OF POTENTIAL THERAPEUTIC MOLECULES DERIVED FROM MEDICINAL PLANTS FOR ROLE IN CANCER AND INFLAMMATION

MAJOR PROJECT REPORT

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CERTIFICATE

This is to certify that the M.Tech dissertation entitled " **Elucidation of potential therapeutic molecules derived from medicinal plants for role in cancer and inflammation**" in partial fulfilment of the requirement for the award of the degree of master of Technology, Delhi Technological University(Formerly Delhi college of engineering, university of delhi), is an authentic record of the candidate's own work carried. The information and data enclosed in this dissertation is original and has not been submitted elsewhere for honouring of any other degree.

CANDIDATE'S DECLARATION

I, BhaskarVerma student of M.Tech Bioinformatics bearing roll no 2K/BIO/02 at the Department of Biotechnology hereby declare that the work entitle "**Elucidation of potential therapeutic molecules derived from medicinal plants for role in cancer and inflammation** " has been carried out by me under the guidance of **Dr. Asmita Das,** assistant professor at Delhi Technological University,Delhi

This dissertation is part of partial fulfilment of requirement for the degree of M.Tech in Bioinformatics. This is the original work and has not been submitted for any other degree in any other university.

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TABLE OF CONTENTS

LIST OF FIGURES

LIST OF FIGURES

LIST OF TABLES

LIST OF ABBREVIATIONS

PDB	Protein data bank
MMP	Matrix metalloeptidase
TLR	Toll like receptor
COX-	Cyclooxygenase
LOX	Lysyl oxidase
VEGF	Vascular endothelial growth factor
PPAR	Peroxisome proliferator receptor
HO-1	Heme oxygenase-1
CBP	C/EBP binding protein
TNF-	Tumor necrosis factor
ICAM	Interracellular cell adhesion molecule
CSF	Colony stimulating factor
STAT	Signal transduder and activator of transcription

ABSTRACT

A number of active compounds from medicinal plants have been shown to possess anti-cancer and anti-inflammatory properties. The active compound obtained from these medicinal plants that exhibit anti proliferative and anti-inflammatory activities were used to check their interaction to the target molecule using AutoDock 4.2. The molecular targets such as TNF-α, chemokine receptor-4, urokinase type plasminogen activator , heme oxygenase-1, involved in cancer and in inflammation are taken from the literature. 28 natural compound are selected for docking studies ligand including butrin, isobutrin, hyperoside, rutin, nortracheologenin withaferin A . These targets binds to their individual target in most of the cases in (table 2) iGEMDOCK virtual screening and interaction tool. Further docking of these screened compound is performed with the target molecule at their active site region. The co-ordinate for active site selection were choosed according to the ligand interaction(PDB) . From the docking results (table 2) compound 1alu_butrin, 3v99_rutin, 2f4b_withanolide A, 1ejn_isobutrin, 1ejn _cur, and 3tgn_hyperoside shows their free energy of binding -4.04, -8.12, -13.51, -7.91, -5.72, -4.04, -3.59 kcal/mol respectively. Out of them the cluster 1alu_butrin and 2f4_withanolide A and 3tgm_hyperoside are selected as good binder according to their estimated free energy binding and reference RMSD value.

PART I

INTRODUCTION

1 INTRODUCTION

Medical plants have been traditionally used as therapeutic target in variety of diseases using plant component as a active target. Use of natural component as active target has many benefits as they have less side effects in human body. Cancer is one of the major cause of death worldwide. The use of natural compound over chemotherapeutic is much of interest as they can reduce adverse side effects occur dunring chemotherapy. Number of plants active compound are reported to have a role in cancer as well as in modulation of other molecular mechanism of the human body. One more reason to to use natural is that they may contain number of active compounds that can act on differents molecular targets and that too in a single plant. The active component from plant curcuma longa shown to posses anti proliferative, anti inflammatory, antibacterial, antifungal, antioxidant properties. Inflammation is response of the body to various outside factors or interanal factors. Inflammation involves is a complex physiological and various reaction in the body causing different such as pain or redness. Due to long persistence of inflammation many diseases arose including cancer and other. Major cells including basophils, mast cells, andphagocytic neutrophils and are involved in the process of inflammation . The inflammatory proces ensures recruitement of appropriate leukocyte that are controlled by cytokines, prostaglandins and other molecule. The mediators discovered has an immense role in disease condition , revealing the complexity of inflammation. It has also increased our better understanding the inflammation.

The knowledge of the molecule involved in cancer and inflammation basis leads to finding of less lethal chemoprotective and anti-inflammatory molecules and agents. In recent years considerable attention has been given to subastance involved in carcinogenesis and also in inflammation related cancer using plant as active targets. The mechanisms of action of many plants component are yet to be understand well however number of plant active component are reported to be involved in cancer and inflammmation.

In this report, the binding mode of compounds which are treated active in medicinal plants, were studied . For this study a graphical user interface program , the the AutoDock 4.2 program. The binding mode of active molecules and their potential target is studies using the

tool. The docking were performed on molecular targets which are found to be common in both cancer and inflammation cyclooxygenase, NOTCH-1, Aryl hydrocarbon receptor, C/EBP homologous protein, Peroxisome proliferator receptor gamma Beta-catenin , chemokine receptor-4, 5-LOX. ICAM , VCAM, Urokinase type plasminogen activator, TNF-alpha, IL-6, Caspase-3 ,which have some role in modulation inhibition regulation of molecules and signalling pathways involved in cancer and inflammation. AutoDock program evaluation binding energy through precalculated grids of affinity potentials by using genetic and lamarkian algorithms in this study to find proper binding.

For this study 28 plant derived active ingredients from different plant medicinal sourece are selected. These compound are screened for particulat targets . Active compounds that binds best screened is then selected for molecular docking using AutoDock4.2.

PART II

REVIEW OF LITERATURE

2 REVIEW OF LITERATURE

The anticancer and anti inflammatory properties of plants are known from long time. According to the National Cancer Institute (NCI) screening of 35,000 plant type for potential anti-inflammatory and anticancer activities along with the knowing interaction analysis. About 3,000 plant species have demonstrated reproducible anticancer activity. Many of these plants natural compound involved in carcinogenesis are also involved in inflammation simultaneously as there evidences that links inflammation leading to tumorogenesis. Many studies have focused on the chemoprotective and inflammatory properties of plants such as anticancer and anti inflammatory properties of medicinal plant *curcuma longa* , which is used in india from thousands of years as a medical remedy.

2.1 The link between cancer and inflammation

In tumour progression inflammation is a critical component it has been seen that many cancer arise from chronic irritation site of infection and inflammation. The link between cancer and inflammation was made known long ago in past century butt not of much interest. This link between cancer and inflammation on was made on the basis of epidemiological studies of patients. Epidemiological studies have led the relation between inflammatory cells and cancer the site of and other presence of inflammatory cells and other small molecules such as chemokines cytokines and prostaglandins in related tissue share link b many relations has been made in relation to cancer and inflammation. It is now clear that cell proliferation alone does not lead to cancer but along with inflammatory cells and DNA damaging agents.

Cancer and inflammation are related by means of extrinsic pathways and an entrinsic pathway. One pathway in which inflammatory contidion increases cancer risk and another in which genetic alterations takes place causing inflammation. Number of molecules such as harmones, enzymes cytokines transcription factor are involved in both cancer and inflammation. One of the major inflammatory cytokines; cyclooxygenase 2 (COX2), which is commonly expressed by cancerous cells express many molecules in relation to inflammation, for eg. on of the major inflammatory cytokine called as cyclooxygenase 2 (COX2) is frequently expressed in tumorogenesis . Cyclooxygenase is involved in the production of

prostaglandins; chemokines that can fascinate monocytes and dendritic cells. Other chemokines that stimulate angiogenesis such as IL-8 and the chemokine receptor CXC-chemokine receptor 4 (CXCR4), which binds to CXCL12; extracellular-matrix-degrading enzymes; and the adhesion molecule lymphocyte selectin (L-selectin).[1]

2.2 Oncogenes and cancer-related inflammation.

A class of oncogenes codes protein tyrosine kinases that are importunately activated in a ligand-independent manner as a result of chromosomal rearrangement or gene mutationtional events. RET is characteristic of these activated oncogenes. A chromosomal rearrangement that affects The RET is affected by chromosomal rearrangement present in a frequent early event in the pathological process of the human papillary thyroid carcinoma. In short-term culture maintainance of human thyrocytes, RET actuates an inflammatory transcriptional program, the molecular components of which are found presented in tumours obtained from samples of the patients. The inflammatory mediators that are produced together with their effects on inflammatory cells and tumour cells, are CC-chemokine ligand; CSF, colony-stimulating factor, CXC-chemokine ligand 12, CXC-chemokine receptor 4, interleukin 8; L-selectin, MMP, matrix metalloproteinase, urokinase-type plasminogen activator, lymphocyte and selectin;.

The production of inflammatory mediators can also be regulated by Tumour-suppressor proteins. such examples of proteins are von Hippel-Lindau tumour suppressor (VHL), phosphatase and tensin homologue (PTEN)16–20 and transforming growth factor-β (TGF-β). VHL is a constituent of a molecular complex that aims the transcription factor called as hypoxia-inducible factor 1α (HIF1α) for degradation. HIF1α upholds the cellular and such as tissue response to hypoxia, including angio genesis. It also interacts with the transcription factor nuclear factor-κB (NF-κB), resulting in the production of the major inflammatory cytokine present in cancer and inflammation both; chemokine receptor CXCR4 in human renal carcinoma cells and the tumour necrosis factor-α (TNF-α) also in other malignant cell types such as type2, type16, type19, type20. The production of Chemokine receptor 4 is particularly significant, because the expression of chemokine receptor 4 is frequently upregulated in human cancer. Evidence from a recent mouse model of cancer links a tumour suppressor protein, TGF-beta that is frequently intricate in the progression of human cancer, and also involved in tumour-promoting inflammation. Breast carcinoma animal model suggests, inactivation of gene encoding type II TGF-β receptor which initiates

carcinogenesis by inhibiting the actions of TGF-β) lets the production of chemokine receptor 5 and chemokine receptor 12. The myeloid-derived suppressor cells (MDSCs) are attreacted ny these chemokines, these cells belonging to the myelomonocytic lineage. myeloid-derived suppressor cells are potent suppressors of adaptive immune responses to tumours and to facilitate metastasis directly.

It willbe important to assess whether this pathway occurs in human tumours in which the TGFβ receptor is involved. Thus, the various types of oncogene (such as those e ncoding protein tyrosine kinases, RAS and RAF, transcription factors and tumoursuppr essor proteins), irrespective of their molecular class or mode of action, all coordinate inflammatory transcriptional programs. And these oncogenecoordinated inflammatory re sponses seem to have aspects in common: a link to angiogenesis, and the recruitment of cells of myelo monocytic origin. Several issues remain to be fully elucidated, incl uding which components of inflammation are essential and which are redundant, the r elative importance of these components in carcinogenesis in different tissues, and the relevance of these components to different types of cancer in humans.

2.3 Evidence that links cancer and inflammation -

1. Signalling pathways involved in inflammation operate downstream of oncogene mutation.
2. Presence of inflammatory molecules at the site of tumor progression.
3. Many small molecules such as chemokines, cytokines and transcription factors decreases the occurrence and outspread of cancer.
4. Overexpression of inflammatory agents (cytokines) stimulate development of tumors.
5.

2.4 Key factors in cancer-related inflammation

In the collection of molecules involved in cancer related inflammation key intrinsic fa ctors such as transcription factors (NFκB ,STAT3), chemokines, major cytokines (IL-6,TNFα and IL1β) can be identified. The inflammatory cytokines NFκB is a key coor dinatorof innate immunity and inflammation, and has emerged as an important endoge nous tumour promoter4 . NFκB is crucial both in the context of tumour or potential t umour cellsand in the context of inflammatory cells. In these cell types, NFκB operat es downstreamof the sensing of microorganisms or tissue damage by the Toll like receptor

MyD88 signalling pathway, and by signalling pathways that is mediated by the infla mmatory cytokines TNFα and IL1β. In addition, can be initiated as a result of amplif ication, mutations or deletions in tumour cells at genetic level. At risk of transformati on by carcinogens, in inflammatory cells in as well as in epithelial cells and tumour cells, activates the expression of genes for adhesion molecules, inflammatory cytokines , enzymes in the prostaglandinsynthesis pathway, inducible nitric oxide synthase and a ngiogenic factors are activated by NFκB. One of the important functions of NFκB in cells targeted by carcinogenic agents is promoting different function ofcell survival, by inducing the expression of antiapoptotic genes (such as BCL2). There is also amassi ng evidence of interconnections between the NFκB and HIF1α systems .NFκB is invo lved in cancerrelated inflammation typically occurs tumour initiation and progression in tissues (such as the gastrointestinal tract and the liver) is by inhibitors that functio n at various stages of the pathway tightly controles the NFκB pathway. Support for t he connection between cancer and inflammationis further strengthened by studies of th e role of NFκB in tumourinfiltrating leukocytes.

There is strong evidence in Cancerrelated inflammation and adaptive immunity from g enetic studies of mouse models that cells of the adaptive immune system can eliminat e nascent tumours by the process called immunoediting. Innate immune responses, ma nifesting as inflammation, are crucial for the initiation of adaptive immune responses. Therefore, divergent effects of inflammation and immunoediting are absurd. Yet, a rec ent study in mice shows that the TLR adaptor MyD88 (which is involved in innate i mmune responses) has a key role in promoting tumour development and that inflamm ationinduced carcinogenesis and immunoediting can occur in the same tumour model . The interplay between adaptive immunity and cancer.related inflammation was shown in studies of a mouse model of cancer that is caused by human papilloma virus. A ntibodies are deposited in the tumour stroma in this system. These antibodies then bin d to unidentified molecules in the ECM and thereby triggering inflammatory responses that stimulate cancer progression58. The differentiation and activation of dendritic cel ls are inhibited by signals present in the microenvironment of tumour. Tumours are fr equently intruded by regulatory T cells, which destroy both adaptive and innate immu ne responses. These cells, and conventional TAMs, are potent suppressors of antitum our immunity. Thus, in cancerrelated inflammation, multiple pathways are effective in

antitumour immunity in established tumours. These pathways, their hierarchy, and wh ether they can be targeted for therapy is still to be be determined well.

Sex steroid hormones intrude a classic, clinically significant pathway of tumour elevati on in breast and prostate cancer and have been a therapeutic target since George Beat son's discovery of hormone dependent breast cancer . Recent studies have uncovered an surprizing relationship between sex steroid hormones and cancer . Macrophages pro duces inflammatory cytokinine interleukin1 beta in the tumour supporting microenviron ment which converts such receptor modulators for their inhibitory action 60. Female s are less exposed to cancer at somesites, such as the liver, which is a not predictabl e target sex steroid hormones organelles. Studies of a mouse model of liver carcinoge nesis, Willscott Naugler et al.61 reported that the sex difference in tumour vulnerabilit y resulted from a downregulation of IL6 production by macrophages. In addition, in male mice, IL6 production was activated to a much greater extent in response to carc inogenmediated tissue damage by activating MyD88dependent TLR and/or interleukin1 receptor signalling pathways). Thus, connections are evolving between the two classic tumourpromoting pathways inflammation and sex steroid hormones. Inflammatory pat hways in invasion and metastasis are studied significantly. Most studies of the mecha nisms of cancerrelated inflammation have focused on the initial stages of cancer, but inflammatory mediators and cells are involved in the invasion, migration and metastasi s of malignant cells. Chemokine receptors and their respective ligands direct the move ment of cells during inflammation, cancer and the maintenance of tissue homeostasis, invasiveness and survival by affecting cell motility . On transformation, many cells st art to express chemokine receptors and thereby use chemokines to aid in their migrati on to, and survival at, sites that are distant from the original tumour. For example, th e chemokine receptor CXCR4 and its ligand CXCL12 are important for cell movemen t in disease states63. CXCR4 is frequently expressed by malignant cells16, and the a mount of chemokine receptor 4 expressed by benign human tumours relates with ext ent to which metastasis to lymph nodes occurs in breast, liver and oesophageal cancer . Other chemokine receptors such as chemokine receptor 1 (CX3CR1), chemokine rece ptor 1 (CCR1), CCR7, CCR9, CCR10, CXCR1, CXCR2, CXCR3, CXCR5 and CXCR 7 are also expressed by tumor cells from a range of tissues and are concerned in org anspecific metastasis; for example, the expression of chemokine receptor 7 correlates with lymphnode metastasis, and expression of CCR9 with metastasis to the small inte

stine. Many of the above receptors are expressed by these malignant melanoma cells possibly explaining melanomas cells are highly metastatic. Expression of chemokine re ceptors is presented by many of the malignant cells for this several mechanisms have been proposed. Autocrine signals and paracrine signals along with genetic and epige netic changes, might contribute to this possible change. For example, the mutation in variable heavy light and the chromosomal rearrangement which affects RET encourage the expression of Chemokine receptor4 on initiated cells. Regardless of the posiible mechanism, it is clear that attainment of chemokinereceptor expression is a very com mon attribute of malignant cells that do not normally express these receptors epithel ial cells and mesenchymal cells even at the early stages of malignancy. The invasiven ess of malignant cells can escalate in the presence of inflammatory cytokines such as TNFα, IL1β and IL6 as a result of the upregulation of chemokinereceptor expression prompted by these cytokines73. For example, autocrine signalling mediated by TNFal pha upregulates expression of functional chemokine receptor 4 by ovarian cancer cells 74, and stable knockdown of mRNA encoding this cytokine diminishes the expression of CXCR4 and its ligand CXCL12 both by the malignant cells. Colonization of the peritoneal cavity, angiogenesis and spread to sites distant from the peritoneal cavity in hibited by such events73. Epithelialmesenchymal transition by breast cancer cells invol ves stimulation of mesenchymal transition done by TNFα being a potent stimulator, a nd also in activation of NFκB signalling. A. Link between NFκB signalling and meta stasis was obtainedmice experimental study of genetic model of prostate cancer. Met astatic spread was found to be reduced by inactivation of gene encoding a major com ponent of the NFκB signalling pathway . The mechanism behind this was found to i nvolve in the activation of receptor activator of NFκB (RANK) in malignant prostate epithelial cells (paracrine signalling). It would be exciting to determine whether 440 I NSIGHT REVIEW NATURE|Vol 454|24 July 2008 chemokine ligands and receptors a lso do contribute to the effects of IKKα and also to find whether IkKα is associated in other metastatic pathways of tumorogenesis. Other cells within in the tumour mi croenvironment also affect processes in later stages of cancer. Inflammatory macropha ges escalate dissemination of tumour cells and spread of metastasis in an ovarian c ancer model. The ability of macrophages to aid in ovarian tumourcell migration and i nvasion can also be modelled in vitro as Coculture of macrophages with tumour cells was shown to rise their invasiveness in an NF-κB-dependent and TNF-α

In summary, Between malignant cells and infiltrating leukocytes autocrine and paracri ne interactions coordinates chemokines and cytokines. These interactions are involved i n increase of the migration, invasion and continued existence of malignant cells. They also affect growth of the primary tumour condition and the known ability of t he tumour cells to inhabit the metastatic place.

2.5 Cancer-inhibitory inflammation

Numerous experimental results and clinical results shows inflammation having protumour activity although some evidence does come into this general pattern. As an example, to such resultd chronic inflammatory response such as that in psoriasis, developing cancer is not associated with an increased risk but it subset cancer. In certain tumours the presence of inflammatory cells is related with better diagnosis such as eosinophils and TAMs. Such observations tells that inflammatory cells can destroy tumour cells as well in addition to normal cells41. For this example is appropriately activated macrophages, a important component of cancer-related inflammation. These activated macrophages can remove and kill tumour cells eliciting cancer-destructive inflammatory responses on the wall ofblood-vessel. In many of the cases their tumour-promoting properties win out 41. The balance between the protumour activities and antitumour properties of macrophages is mainly due to NF-κB 37,83, so by this NF-κB could be targeted to allocate tumour-promoting macrophages towards their antitumour function.

2.6 Unsolved question in relation to cancer and inflammation

The link between inflammation and cancer is accepted in general, but there are several questions eamain to be solved. Some of these questions are-

1. In cancer development, inflammation is sufficient ?

2. which cancer-related inflammatory molecules are common to all al type of tumor environment beside the diversity of tumours and oncogenic pathways.

3. To balance between ‘bad’ inflammation and ‘good’ inflammation? And how it can be altered to favour of immunity instead of tumour promotion?

4. What is the association between MDSCs and TAMs?

5. What is the clinical applicability of the connections in sex steroid hormones to inflammation?

6. How to target in the best way to treat cancer-related inflammation in patients with cancer? being the most important and difficult question.

The properties of some plant and the common molecules involved in cancer and inflammation are included in this study are are described below-

2.7 MEDICINAL PLANTS

- **Curcuma longa**

The main ingredient of about every of Indian food , the popular known as Haldi or haridra has been tradionally used as healing agent. Research into this compound has came to known about its proven effect in immunity.

The active compound 'curcumin' from the extracts of *Curcuma longa(haldi)* ex hibits powerful pharmacological activities. Its medicinal properties have been att ributed mainly to the curcuminoids and the main component present iscurcumin. Curcumin is a active natural component of turmeric (Curcuma longa) and one of the most powerful anticancer and chemopreventive agents. The biological effects of curcuma longa range from antioxidant, anti-bacterial, anti-inflammatory to inhibition of angiogenesis. This medical plant also shown to hold specific antitumoral activity. The varied cellular effects and molecular mec hanism has been studied in details and the plant has been shown to have mul tiple targets and number of interacting macromolecules within the cell.

The antiproliferative properties of curcumin may be related to its ability to do regulate the expression of a number of genes, including NFkappa B, matrix me tallopeptidase 9 (MMP9,Epidermal growth receptor 1 (EGR1), Activator Protein (AP1), cycloxygenase 2 (COX2), nitric oxide synthase (NOS), lysyl oxidase (LOX), and tumor necrosis factor (TNF). Turmeric moderates the expression o f various chemokines, , cyclins and growth factor receptors, including epiderma l growth factor receptor (EGFR cell surface adhesion molecules), and human e pidermal growth factor receptor 2 (HER2). Its effects on gene expression is th at it inhibits the activity of cJun terminal kinase, protein serine/threonine kinas es and protein tyrosine kinases. Turmeric shown to hinder tumor cell invasion andmalignancy by inhibiting HEp2 (epidermoid carcinoma cell line) cell inva

sion and by reducing MMP-2 activity .

Nanoparticles of curcumin has been created recently that are soluble in water which was previously not possible.

- **Targets of curcumin**

Curcumin is shown to interacts with number of targets either by modulating their activity or by binding directly to the target or indirectly regulating their function.More than 30 different proteins have been found to interact with curcumin directly including focal adhesion kinase(FAK), DNA polymerase, thioredoxin reductase, tubulin and protein kinase C,lipoxygenase(LOX) and. Curcumin can also bind to divalent metal ions such as Fe,Cu,Mn and Zn.

- **Transcription factors**

The transcription factors affected in inflammatory and cancer , might be inhibit ed or activated depending on the particular target. Curcumin potently inhibits t he activation of some transcription factors including nuclear factor-κB (NF-κB), , signal transducer and activator of transcription (STAT) proteins, activate d protein-1 (AP-1) , hypoxia inducible factor-1 (HIF-1), early growth response-1 (Egr1) Notch1 and βcatenin, but it also activates other transcription factors such as aryl hydrocarbon receptor (AhR), C/EBP homologous protein (CHOP), electrophile response element (EpRE), (PPARγ) , and NFE2.It has been shown that the nuclear factors, AP1, βcatenin , NFκB, STAT3, HIF1 and Notch1, are involved in cell proliferation, invasion, cell survival, angiogenesis, tumorigene sis and inflammation. In most cancers, these type of transcription factors are fr equently upregulated Curcumin downregulates Notch1 signaling, which resulting in the inactivation of NFκB activity contributimg to cell growth inhibition an d apoptosis in pancreatic cancer cells**.** Nrf2 activation by curcumin has been li nked to the stimulation of hemeoxygenase-1 (HO-1).

- **Growth factors and protein kinases**

Growth factors and their receptors play a critical role in the normal process of growth and differentiation. Unregulated expression of these molecules can lead to abnormal growth and development, resulting in malignant transformation. In addition, increased expression of growth factors, such as transforming growth factorα (TGFα), can lead to nonneoplastic disorders like psoriasis . Curcumin h as been shown to modulate the expression and activity of these growth factors, thereby exhibiting antiproliferative, antiinvasive and antiangiogenic effects . Ch emokin receptor 4 (CXCR4), also called fusin, is an alphachemokine receptor s pecific for chemokine (CXC motif) ligand (CXCL) 12 (str-omal-derived-factor-1, SDF1). It has been shown that the CXCL12CXCR4 axis is involved in seve ral problematic diseases, including cancer cell metastasis, leukemia cell progress ion and rheumatoid arthritis.Thus, CXCR4 is thought to be one of the greatest therapeutic targets to overcome the above diseases**.**

- **Inflammatory cytokines**

Excessive synthesis and production of proinflammatory cytokines during severe infection or injury, including TNFα, IL6 and IL1β , play important role in dev elopment of local as well as systemic inflammation, causing organ failure and severe pathophysiological derangement. Cytokine gene and activation are tightl controlled in producing cells, as a result of transcription. Therefore, inhibition of proinflammatory cytokine assembly by regulation of NFκB transcription fact or, is an potential approach for monitoring inflammatory responses. Studies hav e demonstrated that curcumin modulates the production of inflammatory cytoki nes, exhibiting a potent anti-inflammatory activity**.**

In systemic inflammation and regulation of immune cells TNFα has an import ant role. The implicatetions of TNFα Dysregulation can lead to inflammatory diseases (such as rheumatoid, multiple sclerosis, arthritis , Crohn's disease, ps oriasis and as well as in cancer. Studies showed that curcumin has inhibitory e ffects in the production of TNF-α.

- **Enzymes**

In inflammation and cancer variety of associated enzymes were found to be modulated by curcumin including 5-LOX, COX-2, and phospholipases A2 (PLA2)and inducible nitric oxide synthase (iNOS). The inducible COX enzyme form , can be induced by inflammatory stimuli and mitogenic stimuli. As a results of this induction enhanced synthesis of prostaglandins takes place. Some of the evidenced showed that COX2 is overexpressed in a variety of human cancers, such as liver, brest, bladder, colon, pancreas, lung, skin, stomach, head and neckcance rs. Pharmacological inhibition of COX2 protected against the development of tumors in animals. Curcumin can downregulate the expression and the activity of COX-2.

- **Adhesion molecules**

CAMs are glycoproteins present on the surface of cells**.** In the process called cell adhesion, cell adhesion molecule (CAMs) binds with extracellular matrix and other cells on the surface. Cell surface expression of adhesion molecules, such as ICAM1, VCAM1, and endothelial leukocyte adhesion molecule1 (ELA M1) plays a critical role neoplastic diseases and inflammation. It has been testi fied that inhibition of NF-κB blocked TNF-α-induced expression of ICAM-1, VCAM1, and Eselectin completely, indicating that expression of cell adhesi on molecules is little regulated by NF-κB.

The integrins are heterophilic cell adhesion molecules that bind immunoglobulin superfamily CAMs or the extracellular matrix. During the last decade, studies on the function of integrins regulate an array of cellular processes, including proliferation, cell death, migration and differentiation.

- **Apoptosis-related proteins**

A mechanism of cell death occurring after enough cellular damage to cell th at is apoptois. This process is essential for the development and the maintena

nce of cellular homeostasis in unicellular and multicellular organisms. Deregulat ion in the process of apoptosis can lead to autoimmune, degenerative diseases and cancer. Therefore, elucidation of the pathways involved in disease etiology and the identification of compounds that can induce cell death has an increasin g interest. Demonstrated studies shows that curcumin can induce apoptosis, and inhibit the tumor initiation and promotion in animals. The ability to induce ce ll death is due to the chemopreventive action of curcumin in many of the path ways. A microarray results showed that t among the 214 apoptosis.associated g enes the expression of 104 genes was altered by curcumin. The upregulated ge nes by curcumin included TRAF6, CASP14, HPRT, GADD45, NIP1, MCL1, BCL2L2, GSTP1TRAP3, DAXX, UBC, PIG11, PIG3, CDC10, PCNA, , JNK1 and RBP2. The downregulated genes were TRAIL, TNFR, AP13, IGFBP3, SA RP3, PKB, IGFBP, CASP7, CASP9, TNFSF6, TRICK2A, CAS, TRAILR2, RA TS1, hTRIP, TNFb and TNFRSF5. Targets of curcumin have been discovered i n a signified amound in recent years involving the signaling pathways implicat ed in apoptosis.

- **OTHER TARGETS**

The other molecular targets include HSP 70, cyclin D1, DNA fragmentation factor 40-kd subunit, urokinase-type plasminogen activator (uPA), multi-drug resistance protein(MRP), and uPA receptor. Targeting these proteins contributes to the therapeutic effect of curcumin as these proteins are important for cell proliferation, growth, migration, survival, invasion and many other cellular function including resistance to drug.

- **<u>WITHANIA SOMNIFERA(ASHWAGANDHA)</u>**

W. somnifera has been in use in the Indian traditional system of medicine for ages for its energy-promoting and anti-stress benefits.

It has been reported earlier that Th1immune upregulation is mainly effect of the root constituent withanolide A. Another chemical constituent of

W. somnifera is Withaferin A which is mainly distributed in leaves and produces apoptosis in cancer cells. Synthesis of these withanolides and their isolation from the plant in therapeutic amounts has posed limitations for their effective utility.A formulation have been devised by Scientists at Indian Institute of Integrative Medicine, Jammu, India having a unique novelty where the mixture of leaf and root in a certain ratiowas prepared to obtain a pharmaceutical composition rich in both plant compound withanolide A and withaferin A [IndianPatent: 0202NF2006; Del 01321 dated 19062007]. The formulation offer a action against cancer disease as supported by their current studies. The formulationof this plant has been shown to persuade cell cytotoxicity in cancer cell lines. The suggested for this mechanisms of cytotoxicity include activation of both intrinsic apoptotic signalling and extrinsic apoptosis signaling cascades, activated by generation of nitric oxide (NO) and reactive oxygen species (ROS) in cancer cells. High content of withaferin A formulation in this plant suggests cell signaling pathways.

- **CEDRUS DEODARA**

Cedrus deodara (Roxb.) also called as deodar , is a species of cedar native to the North-Central India, Western Himalayas in Eastern Afghanistan, Northern Pakistan, Western Nepaland South Western Tibet. The chemical active constituents obtained include isopimpillin, wikstromal, dibenzylbutyrolactol, matairesinol(used as ligand), berating, lignans, 1,4 diaryl butane, isohemacholone, deodarone, deodarin, β-himacholone, deodardione, limonenecarboxylic acid, α-pinene, myrcene, β-pinene, cedrin, atlantone, taxifolin, dihydromyricetin cedeodarin (6-methyltaxifolin), and cedrinoside. In traditional system various parts of this plant are used in medicine for the treatment of apoptosis, inflammation, fever, pain, spasmodic, ulcer insomnia, hyperglycemia, disease of skin and blood infections, disorder of mind,. Recent studies indicates its anti-apoptotic, anti-cancer, anti-inflammatory, analgesic, anti-hyperglycemia, insecticidal, antispasmodic, immmunomodulatory properties.

- **TINOSPORA CORDIFOLIA**

Medicinal value of natural product are gaining importance due to their well-known property of no side effects as compared to drugs. The common name of *Tinospora cordifolia* is “Guduchi” which is known for its application in treating diseases. The discovery of active components from the plant and their biological purpose in disease control has led to active interest in the plant.

- **LYCOPERSICUM ESCULENTUM**

All green parts of the plant are poisonous. This plant possess health benefits such as cancer preventive properties and cardiovascular properties by downregulating the inflammatory response. Inflammatory mediators such as reactive oxygen species are inhibited andalso the inhibitionof inflammatory cytokines which changes the expression of cyclooxygenase and lipoxygenase.

- **ANDROGRAPHIS PANICULATA**

Andrographis paniculata also known as kalmegha in india its extract is traditio nally used as a medication to treat different associated diseases in India, China and Southeast Asia. It is an annual herbaceous plant it is also planted in some parts of Europe. A. paniculata is mainly used to get rid of body heat, removi ng toxins from the body these include prevention to common cold, respiratory tract infections including fever It is also used an antidote against poisons of sn akes and insects . According to the studies the plant has been reported to exhi bit various type of biological activities such as antiinflammatory anticancer, ant ibacterial, antiviral, immunomodulating/ immunostimulatory anti HIV (Human-immunodeficiency virus) ,

The plant showed potential therapeutic action in common cough and colds in h uman curing liver disorders. The secondary metabolites present in this plant h ave charatersticly enhanced its importance in the area of medicinal plants.

- **BOSWELLA SERRATA**

The plant resins has been used in medicines since long time. *Boswellia serrat a* (*Salai/Salai guggul*), is a moderate to large sized branching tree of family B urseraceae (Genus Boswellia), grows in dry mountainous regions of India, Nort hern Africa and Middle East. Gumresin extracts of the plant *Boswellia serrata* have been traditionally to treat various chronic inflammatory diseases. The res inous part of *Boswellia serrata* possesses tetracyclic triterpenic acids, monoterpe nes, triterpenes, diterpenes, and four major pentacyclic triterpenic acids i.e. β-boswellic acid, acetyl-β-boswellic acid, acetyl-11-keto-β-boswellic acid and 11-ketoβboswellic acid are responsible for inhibition of proinflammatory enzymes. Acetyl11ketoβboswellic acid of its four major components is most potent inhibi tor of 5-lipoxygenase, which is a enzyme responsible for chronic inflammation.

Table1: **medical plants and their active ingredients**

Sr.no	Plant	Active compound
1.	Curcuma longa	i. Curcmin ii. Dimethoxy curcumin iii. Bis demethoxycurcumin iv. Cyclocurcumin
2.	Withania somnifera	i. Withanolide A ii. Withaferin A(componentA)
3.	Cedrus deodar	i. Matairesinol ii. Nortracheologenin iii. Di benzylbutyrolactolligan iv. Gamma linolenic acid
4.	Tinospora cordifolia	i. 20,beta-hydroxyl ecdysterone ii. Cordioside iii. Columbin
5.	Lycospersicum esculentum	i. Naringenin
6.	Evening primrose	i. Gamma linolenic acid
7.	Butea monosperma	i. Isobutrin ii. Butrin iii. Butrin isobutrin
8.	Buck wheat	i. Rutin ii. chlorogenic acid iii. hyperoside

9.	Andrographis paniculata	i. 5-hydroxy-7-8 dimetoxyflanone ii. Andrographolide iii. Sitosterol iv. Ergosterol
10.	Boswella serrate	i. Boswellic acid ii. 3-o-acetyl-11-keto beta-boswellic acid
11.	Rosemary	i. Rosmarinic acid ii. ursolic acid iii. oleonolic acid
12.	Prunella vulgaris	i. Rosmarinic acid ii. Betulinic acid iii. ursolic acid iv. oleonolic acid

PART III

MATERIALS/ TOOLS USED

3 MATERIALS AND TOOLS

The three dimensional structure of complex 5MU8, 3LN1, 1EJN, 3TGM, 3V99,3LN1, 3ODU are downloaded from PDB database.

Table 2: Macromolecular targets and their pdb ID's

serial no.	Therapeutic target	PDB id
1	NOTCH-1	1TOZ
2	CASPASE3	1PAU
3	TNF ALPHA	5 MU8
5	IL-6	1ALU
6	UROKINASE TYPE PLASMINOGEN ACTIVATOR	1EJN
7	ICAM-1	1P53
8	HEME OXYGENASE-1	3TGM
9	5-LOX	3V99
10	COX-2	3LN1
11	COX-1	1EQG
12	CHEMOKINE RECEPTOR-4	3ODU

PART IV

METHODOLOGY

4 METHODOLOGY

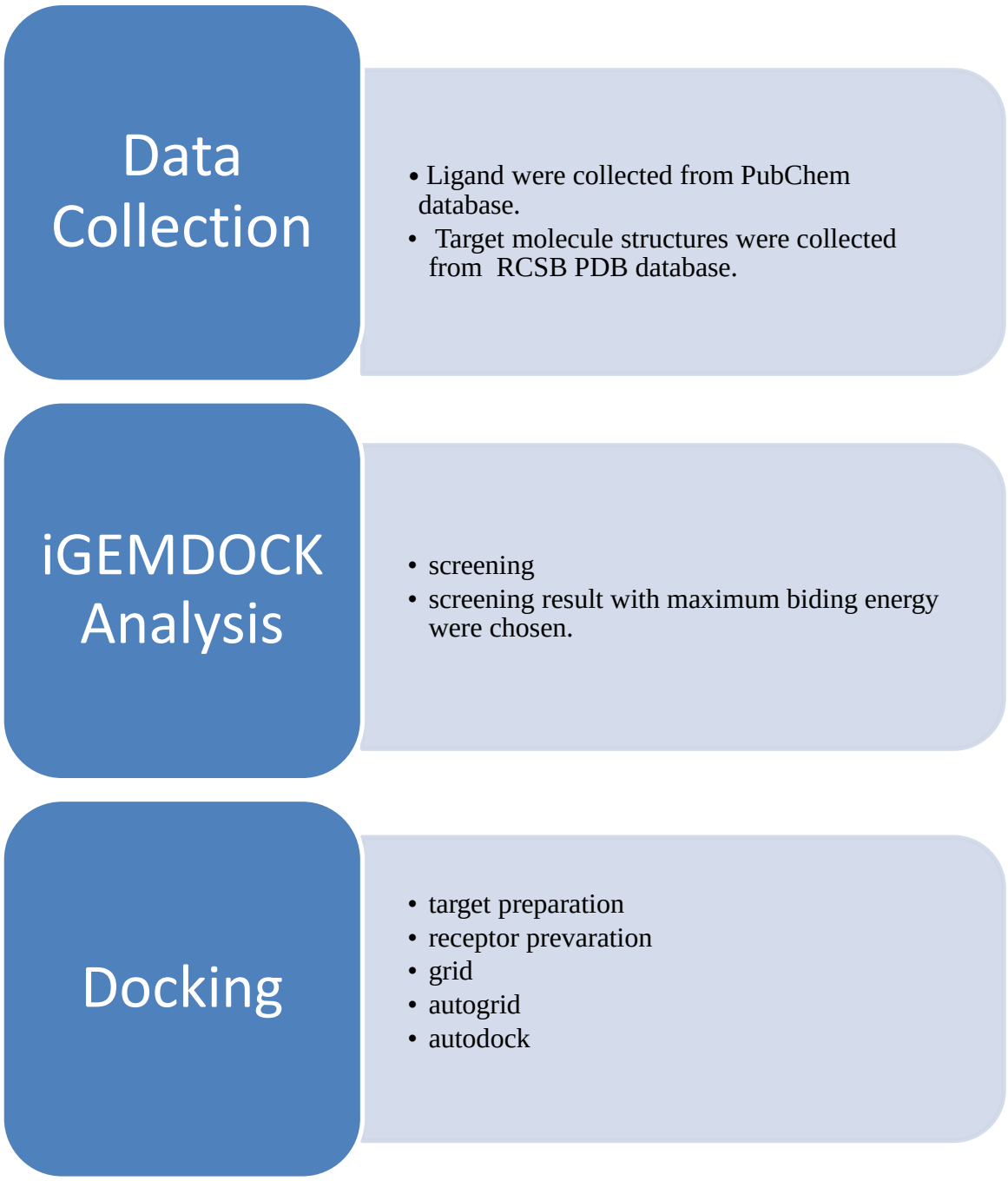

Figure 1: workflow employed in docking

Tools used:

virtual screening and interaction tool

Plant active compound were subjected to virtual screening using iGEMDOCK(A graphical environment for recognising pharmacological interactions and virtual screening). From there active component are obtained having different binding energy and further component having maximum binding energy to their target molecule are selected for docking studies using AutoDock programme from scripps institute(USA).

Autodock 4.2

The structures of 28 plantderived compounds and their conformations were used as st arting conformations to perform docking.

- Ligand preparation

The ligand were prepared using by coverting into .pdbqt file format readable by AutoDock and by detecting torsion tree . The number of torsions are selected by default by the AutoDock programme.

- Receptor preparation

All Xray crystal structures were obtained from the Brookhaven Protein Data Bank (htt p://www.rcsb.org/pdb). Receptors were prepared for docking in such a way that all he teroatoms (i.e., nonreceptor atoms such as water, ions, etc.) were removed. Kollmann charges were assigned. Different parameters were added to the final macromolecule str ucture using the utility of AutoDock

- Grid generation

For grid generation pdbqt file of of liand and pdb files of receptor was selected then grid parameters were selected.the centre of grid box has coordinates XYZ to cover binding pockets that are selected according to the active site presented within the molecule and then AutoGrid run is performed.

- AutoDock4.2 Docking

Polar hydrogen was added and non polar were merged to the ligand moieties along with assigning of Gasteiger type were assigned and the nonpolar hydrogens were merged with the

carbons and the internal degrees of freedom and no of torsions were set. Lamarckian genetic algorithm was extensively used for molecular docking. The parameters such as the mutation rate of 0.02, population size of 150, and a crossover rate of 0.8 were fixed accordingly. The molecular docking Simulations were achieved up to 2.5 million energy and the estimations were maximum at 27000 generations. Each simulation was carried 10 times which yielded 10 docked conformations. The lowest energy conformations were selected as the best binding conformations. In the end, the reverse validation processes ensured the identified hits that fitted with generated pharmacophore models and active sites of both targets. Since all the parameters were required for molecular docking were consequently set as defaults used in regular process of docking.

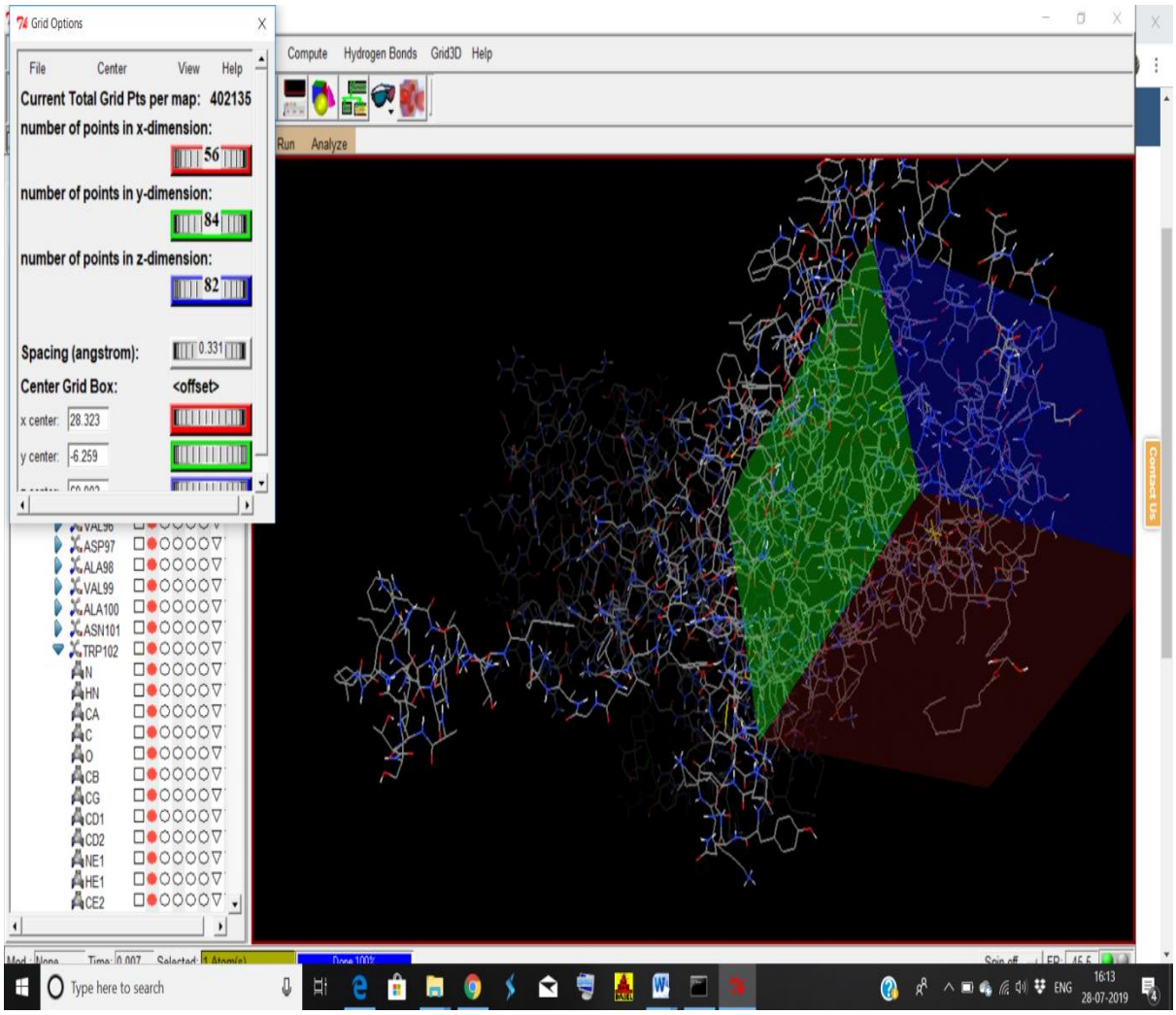

Figure 2: Grid generation using active site co-cordinates

To ensure that the ligand orientations and positions obtained from the docking studies were likely to represent valid and reasonable potential binding modes of the inhibitors, the docking

methods and parameters used were validated by redocking and cross-docking experiments. First, each ligand was docked into the native protein to determine the ability of AutoDock program to reproduce the orientation and position of the ligand observed in the crystal structure.

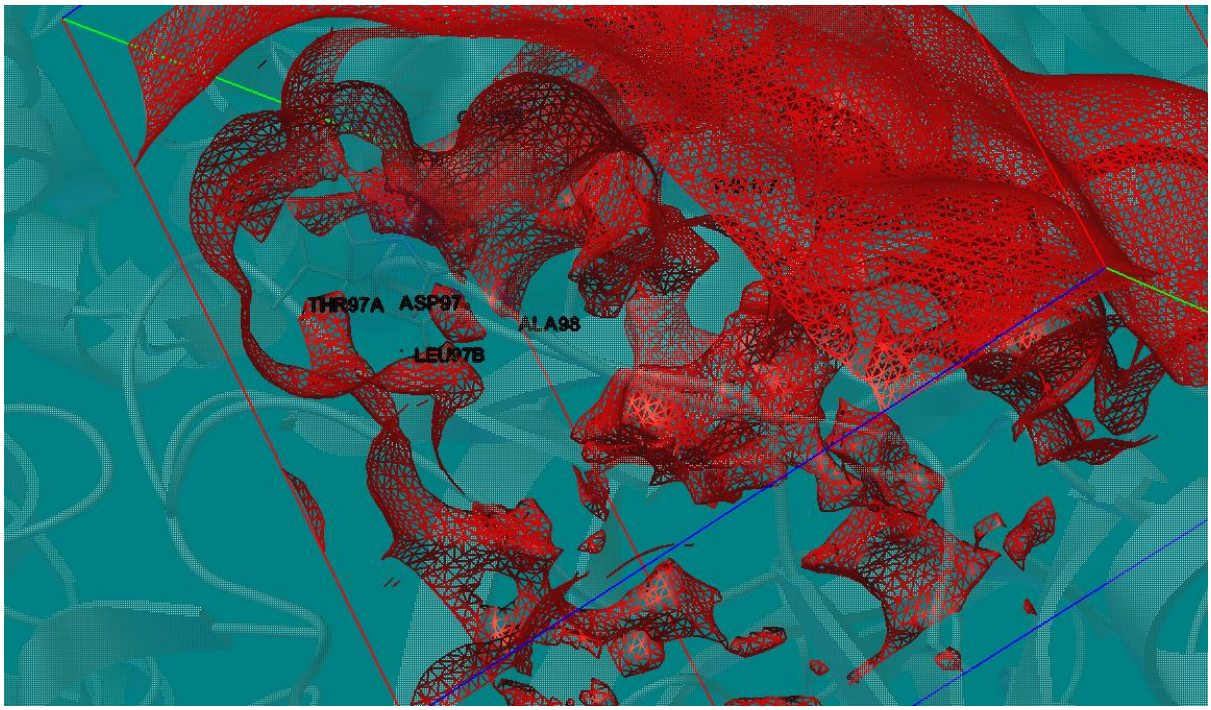

Figure 3: Interaction map of ligand

PART V

RESULTS AND DISCUSSION

5 RESULTS AND DISCUSSION

iGEMDOCK analysis

The input format of receptor structure for iGEMDOCK is PDB format. For the preparation of compounds mol2 file formats of selected ligands are taken and population size of 200 and 70 generation is selected by default. Binding energy calculation is done for each receptor using 28 active compounds. The compound having minimum binding energy were selected for further analysis with AutoDoCk program.

TABLE 3: iGEMDOCK results

	Butrin	**Iso butrin**	**hypero side**	**Rutin**	**Chloro-genic acid**	**Curcum in**	**Rosma r-inic acid**	**withafe rin**
1EJN	**-163.4**	**-150.6**	**-140.6**	**-138.7**	**-134.7**	**-126.6**	**-123.8**	**-115.2**
1PAU	-	**-111.9**	-	-	-	-	-	-
5 MU8	**-134**	**-125**	**-123.7**	**-116.9**	**-113.9**	**-118.2**	**-103.8**	**-102.9**
2AZ5	**-134.6**	**-129.4**	**-123**	**-143.3**	**-103.2**	**-104.9**		**-119.9**
3TGM	**-133.5**	**-135.1**	**-126.6**	**-144.8**	**-120.4**	**-103.5**	**-114**	**-108.7**
3V99	**-135.7**	**-136.4**	**-123.3**	**-142.1**	**-114.2**	**111.7**	**-107.6**	**-113.3**
3LN1	**-106.6**	**-104.4**	**-102.4**	**-104.4**	**-92.2**	**-70**	**-79.5**	**-75.4**
1EQG	**-111.9**	**-104.5**	**-103.2**	**-96.7**	**100.7**	**93.5**	**92.7**	**92.8**
3ODU	**-116.4**	**-119.3**	**-110.7**	**-98.6**	**-98.4**	**-91**	**-93.4**	**-92**

AutoDock Results-

The data of 28 plant derived natural structures obtained from medicinal plant extract is used in this docking study. Docking was performed with AutoDock 4.22 (Scripps Research Institute, USA). Docking to macromolecule was achieved using an free energy function and Lamarckian Genetic Algorithm, with an initial population of 250 randomly placed individuals, a maximum number of 106 energy evaluations, a mutation rate of 0.02, and a crossover rate of 0.80. One hundred independent docking runs were completed for each ligand. Results differing by \2.0 A ˚ in positional root-meansquare deviation (RMSD) were clustered together and represented by the result with the most favorable free energy of binding.

The AutoDock tools gives 10 docked conformations are according do their increasing binding energies. The lowest energy conformations were selected as the best binding conformations. One of the docking interaction of Human TNF alpha to the active compound curcumin with different energy calculations is shown below in Figure4.

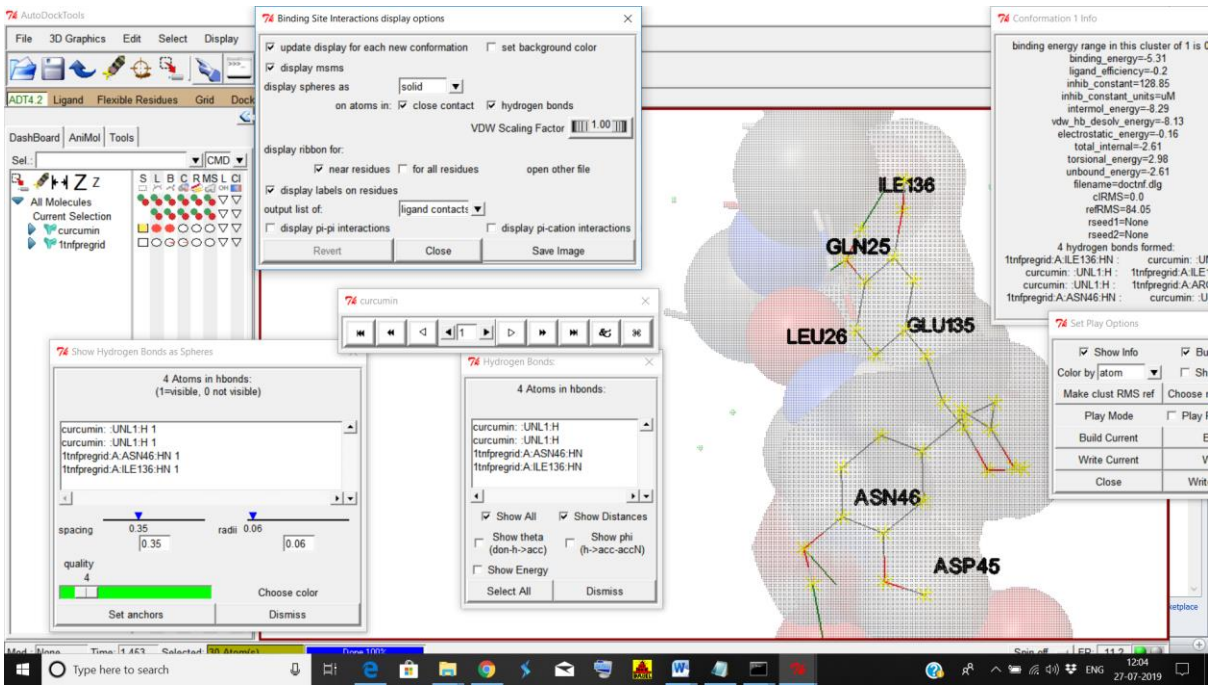

Figure 4: B**inding pocket of 1TNF to curcumin**

3tgnhyperoside

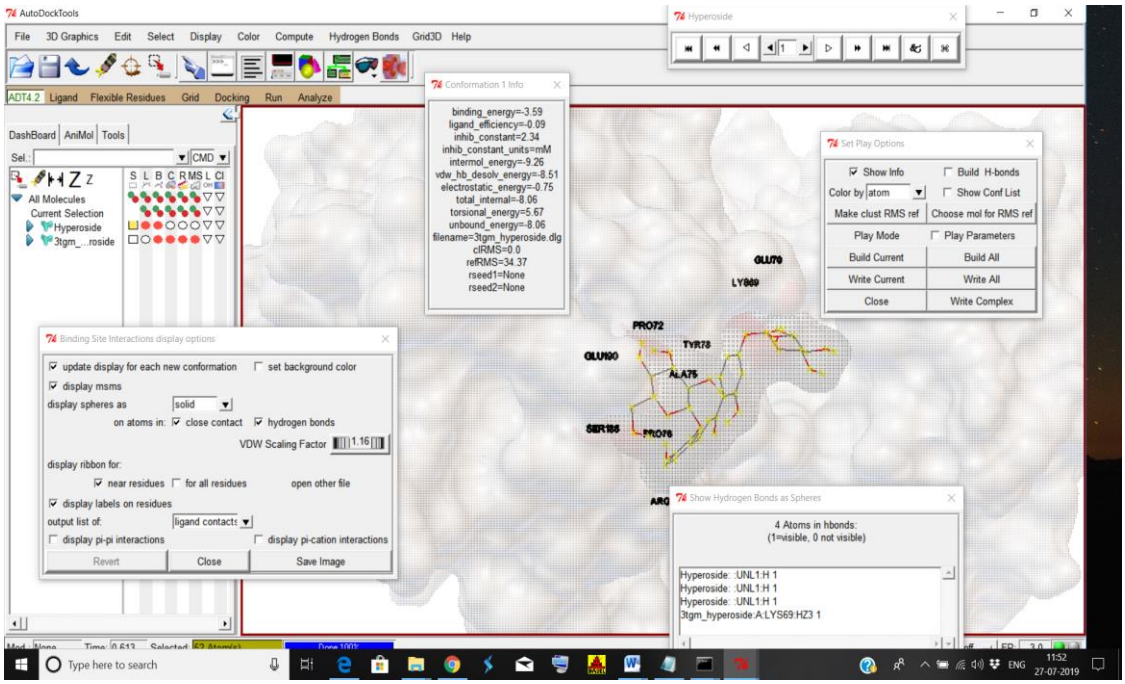

Figure 5: Binding of hyperoside to the target with different parameters such as conformation, bonding, binding site interaction .

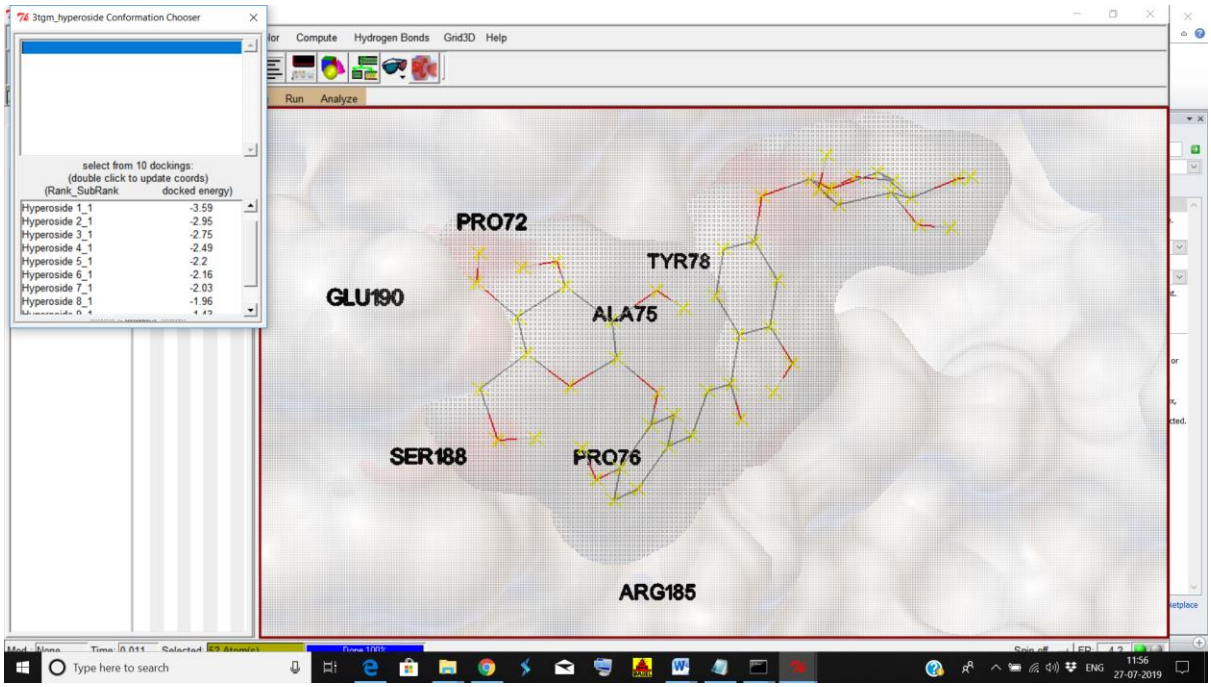

Figure 6: curcumin binding with docked conformation

The docking result of different target molecule and there is summarized here along with the model

Docking poses generated by the AutoDock program poses for ligands in the active sites of target molecule. For each docked pose, information containing the rankwise docking score is displayed on screen above which allows analysis of binding energy relationships. Moreover, results from multiple docking runs are summarized in a table. The docking poses are ranked according to their docking scores and both the ranked list of docked ligands and their corresponding binding poses and their cluster RMSD are summarized below.

TAGET MOLECULE	**MAX BINDING ENERGY**	**CLUSTER REFERENCE RMSD**
1alu_butrin	-6.96	29.93
5mu8_butrin	-8.12	20.08
3v99_rutin	-13.51	94.96
2f4b_withanolide A	-7.91	31.68
1ejn_isobutrin	-5.72	213.52
1ejn _cur	-4.04	54.17
3tgn_hyperoside	-3.59	34.37

TABLE 4: clustering of biding energy of macromolecular target to their active molecule

6 CONCLUSION

The docking studies of targets molecule in cancer and inflammation with the 28 natural compound aims to fit in the target molecule. The presence of ligand molecule in protein structure increases the efficiency of structure based tools so as the ligand molecule may try to fit into the active site of the protein molecule. The binding of ligand molecule to protein shows some likeliness to the drug molecule which were used already.

The result of this study shows that the active molecule such as butrin , isobutrin ,hyperoside withanolide A and curcumin can bind better that other compound chosen. The molecule butrin and isobutrin posses much torsions so they interact with most of the target in drug screening

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