Anticancer activity of *Cassia fistula* Linn through *In vitro* and *In silico* approach

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BY

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DECLARATION

I declare that the dissertation entitled "**Anticancer activity of *Cassia fistula* Linn through *In vitro* and *In silico* approach"** has been prepared by me under the guidance of research supervisor Dr. Vikas Jaitak, Assistant Professor, Centre for Chemical and Pharmaceutical Sciences, School of Basic and Applied Sciences, Central University of Punjab. No part of this dissertation has formed the basis for the award of any degree or fellowship previously.

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CERTIFICATE

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ABSTRACT

"Anticancer activity of *Cassia fistula* Linn through *In vitro* and *In silico* approach"

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ABSTRACT

Cassia fistula L. (Leguminoseae) is a plant species called "Aragvadha" that means "disease killer". *C. fistula* consists of plethora of medicinal properties. A detailed discussion is depicted of the therapeutic potential and chemical composition of *C. fistula* that is responsible for its highly important medicinal properties. *C. fistula* contains many chemical components like anthraquinones, anthocyanindins, proanthocyanidins, flavanoids, polyphenols, alkaloids, saponins, coumarins, tannins, etc. These constituents are reported to possess various biological properties such as antioxidant, antimicrobial, antidiabetic, antitumor, antimelasmic activities etc. In the current dissertation work we limited our study to cancer; specifically, Lung cancer (LC) and Breast cancer (BC) which are spread all over the world to a threatening level. The present study is an effort to contribute to such a multi-targeting moiety from the plant *C. fistula,* on the basis of *in vitro* and simulated analysis. To explore the anticancer activity of *C. fistula*; *in vitro* (cytotoxicity studies) on different extracts and *in silico* studies using MMGBSA (Molecular Mechanics – the Generalized Born model and Solvent Accessibility) on the earlier reported molecules was performed; to study protein – ligand binding interactions on four different targets. The different extracts (Petroleum

ether (SVA-1) (for defatting), Ethyl acetate (SVA-2), Methanol (SVA-3), Hydro-methanolic (SVA-4) Extracts) were prepared in order of their increasing polarity. *In-vitro* cytotoxicity studies done using MTT (3-(4, 5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay on the extracts of plant and their IC_{50} values were calculated. It was performed to identify plant's cytotoxic activity. Targets studied via performing *in silico* protein-ligand binding interactions of majorly mutated yet interlinked pathways of tyrosine kinase and serine/ threonine kinase family; that are namely EGFR, PI3K, Akt and mTOR receptors on which reported molecules of plant were studied. *In silico* studies were performed using maestro 9.6 Schrodinger software. MMGBSA and ADMET analysis were performed to discover and understand protein – ligand interactions between the chosen ligands of *Cassia fistula* and selected kinase receptors. On conducting MMGBSA (Molecular Mechanics – the Generalized Born model and Solvent Accessibility) studies 5,3,4-tri-hydroxy-6-methoxy-7-O-α-L-rhamnopyranosyl-(1→2)-O-β-D-galactopyranoside along with few other moieties illustrated fine binding interactions against standard inhibitors of all the protein targets. From *in vitro* experiment, it was found that Hydromethanolic extract (SVA-4) showed prominent cytotoxicity against MCF-7, A-549 and H-460 cell lines particularly near 50μg/ml of concentration. Results were found in comparable with the NCI criteria limits for IC_{50} values of extracts obtained in assay. Also extracts were stable in DMSO solvent even when kept at the ambient temperature for 30 days and gave consistent results against cancer cell lines. As per the results of the *in silico* studies, the 5,3,4-tri-hydroxy-6-methoxy-7-O-α-L-rhamnopyranosyl-(1→2)-O-β-D-galactopyranoside (dG Bind score -98.7866kcal/mol) for EGFR in comparison to gefitinib (dG Bind score -86.5585kcal/mol); (dG Bind score -87.3524kcal/mol) for Akt as compared to AZD 5363 (dG Bind score -76.5959); (dG Bind score -87.1051kcal/mol) for PI3K as compared to wortmannin (dG Bind score -79.0654kcal/mol) and for mTOR it has shown the (dG Bind score of -81.964kcal/mol) against sirolimus (dG Bind score -192.354kcal/mol) is reported to have fair pharmacokinetic profile along with attractive binding interactions with EGFR, PI3K, Akt and mTOR receptors.

(Akanksha Sharma) **(Dr. Vikas Jaitak)**

(Dr. Sandeep Singh)

DEDICATED

TO

MY BABA AND MY FAMILY

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(Akanksha Sharma)

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LIST OF ABBREVIATIONS

Sr no	Full form	Abbreviation
1.	*Cassia fistula* L.	*C. fistula* L.
2.	Methanolic Extract	ME
3.	Basal Like Breast Cancer	BLBC
4.	Triple Negative Breast Cancer	TNBC
5.	Estrogen Receptor	ER
6.	Progesterone Receptor	PR
7.	Human Epidermal Growth Factor Receptor 2	HER-2
8.	Lung Cancer	LC
9.	Small Cell Lung Cancer	SLC
10.	Breast Cancer	BC
11.	Non-Small Cell Lung Cancer	NSCLC
12.	Epidermal Growth Factor Receptor	EGFR
13.	Phosphatidylinositol-4,5-bisphosphate 3-kinase	PI3K
14.	Phosphatidylinositol-4,5-bisphosphate 3-kinase, Catalytic subunit Alpha	PIK3CA
15.	Phosphatase and Tensin homolog	PTEN
16.	Epiregulin	EREG
17.	collagenase 1 of Matrix Metalloproteineases	MMP1
18.	Matrix Metalloproteineases 2, and Gelatinase A	MMP2
19.	7, 12-dimethyl benz(a)anthracene	DMBA
20.	Reactive oxygen species	ROS
21.	Di-methyl sulfoxide	DMSO
22.	S6 kinase1	S6K1
23.	Eukaryotic translation initiation factor 4E Binding Protein	4EBP1
24.	Eukaryotic translation initiation factor 4E	eIF4E
25.	heat shock protein	(HSP) 90
26.	Thin Layer Chromatography	TLC
27.	Protein data bank	PDB
28.	Extra Precision	XP

29.	Standard precision	SP
30.	Absorption, distribution, metabolism, excretion and toxicity	ADMET
31.	Absorption, distribution, metabolism and excretion	ADME
32.	Glide score	G. score
33.	DiPhenylPicrylHydrazyl	DPPH
34.	Ranikhet disease virus	RDV
35.	Foot and Mouth Disease Virus	FMDV
36.	Lipid peroxidation	LPO
37.	Ethanol leaf extract	ELE
38.	Serum Glutamic Oxaloacetic Transaminase	SGOT
39.	Serum Glutamate-Pyruvate Transaminase	SGPT
40.	Half Maximal Inhibitory Concentration	IC_{50}
41.	Dulbecco's modified Eagle's medium	DMEM
42.	National Cancer Institute	NCI

APPENDIX A

Protein – Ligand binding interactions data on EGFR kinase receptor

APPENDIX B

Protein – Ligand binding interaction data on Akt kinase receptor

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APPENDIX E

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CHAPTER – 1

INTRODUCTION

CHAPTER -1

1. Introduction

Cancer is one of the most prominent killer diseases; it is among the major threats appertaining to all the diseases (Jemal et al., 2011, Wang & Zhang 2012); that are placed in the list of world's top ten life threatening disease. As per, the present statistics of cancer in the world, there is a huge figure of unsuccessful cases and a large number of different types of cancer are being diagnosed in many patients to date (Siegel et al., 2013). An opinion made after a rough assessment; by the American Cancer Society and the International Union Against Cancer indicate that last year 12 million cases of cancer were detected worldwide, out of which mortality was observed in 7 million cases; these numbers are anticipated to raise by twofold by 2030 (27 million cases with 17 million deaths) (Gupta et al., 2009, Bhanot, Sharma & Noolvi 2011). As per the recently surveyed data, some dominating forms of it are prostate cancer, colorectal cancer, lung, breast and cervical cancers (Jemal et al. 2011, Koshki et al., 2013, Siegel et al. 2014). Cancer growth is a consequence of intricate methods that allows a tumor to develop and metastasize. Metastasis is an activity that drifts primary tumor further to other organs and progresses into a metastatic lesion, signifying approx. 90% of all human cancer mortalities. This progression involves step by step migration and establishment of primary cancerous cells as following: (a) indigenous tumor cell multiplication and permeation, (b) (intravasation) access of cancerous cells into the vasculature, (c) (extravasation) migration of these cells via the blood flow to distal tissues and (d) replication and development at the metastatic site. Altogether these points; give upsurge to malignant cancer (Figure 1).

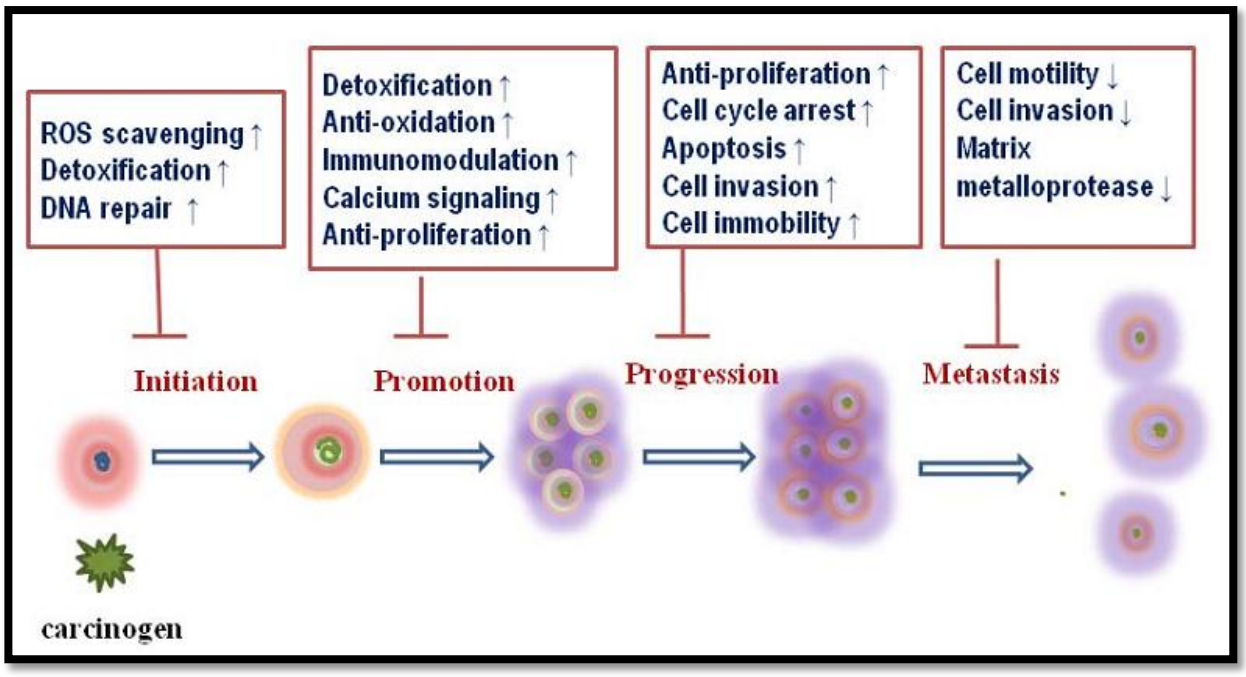


Figure 1: Cancer Progression steps. (Raghu *et al*., 2012)

1.1 Traits of cancer

It is a genetic disease which starts with genetic mutations in various genes which regulates the cell division followed by spreading to other parts i.e. invasion and metastasis (Figure 2). If a single normal cell, start making its clones in an uncontrolled manner, it can be depicted as a fundamental feature of tumour development. Further, mutation following selection of rapidly multiplying cells from the existing population. This then results in the progression of tumour with increasing metastasis via blood circulation to the neighbouring or distant locations. There are six usual traits (or steps) that help the transformation of normal cell to malignant tumour cells, i.e. 1. Normal cells grow and divide on stimulation from growth factors, and when signals from growth factors are not supplied, normal cells commit suicide (undergo apoptosis). While cancer cells do not require growth factor signals to proliferate; 2. Cancer cells do not respond to growth inhibiting signals that come from surrounding matrix, thus inhibiting cell division during Interphase; 3. When the deterioration of the cell takes place either due to damage to DNA, over expression of oncogenes or deficiency of oxygen, these changes are monitored by the sensors like (IGF-1/2) then the cells are programmed to die.

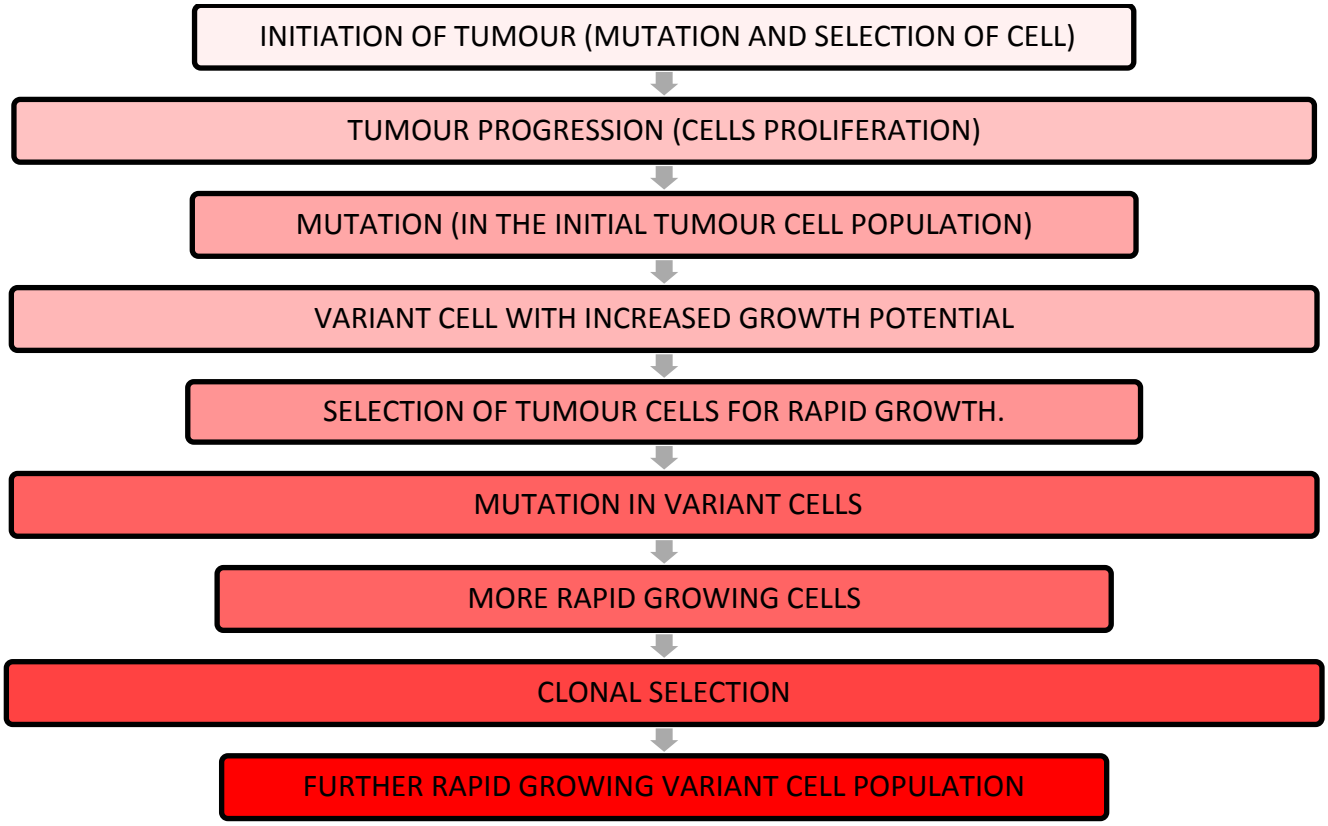


Figure 2: Traits of Cancer.

But cancer cells escape this mechanism; 4. Normal cell's life terminates after certain number of divisions, but cancer cells have the relentless potential to grow and divide (immortally). Telomere analyses the cell multiplication and on completion of each cell cycle it release the DNA at the tip of every chromosome. Many cancer cells maintain the concentration of telomerase enzyme which prevents telomere detachment; 5. Cancer cells undergo Angiogenesis (new blood vessels formation) to supply oxygen and nutrients to its cell population. Loss of p53 tumor suppressor genes may help angiogenesis; 6. Cancer cells originate from a particular site invade into surrounding tissues & then gradually spreads to the different body parts i.e., metastasis. Some new traits were also given by hanahan in 2010 that are, "Energy generation in cancer cells is done by using unnatural metabolic pathways, Body's immune system fails to recognize cancer cells, Progressive mutations in the genes of cancer cells worsen the disease and many types of cancer at times may be induced by local chronic inflammation" (Cooper and Hausman 2000, Hanahan and Weinberg 2011).

1.2 Cancer worldwide status and pathways involved

The dominating proportion that has been spread all around in the patients is of the solid tumors (Gollob et al. 2006). Cancer cell activities are altered by surrounding conditions; metabolism is stalled by the deficiency of nutrients and growth factor, mutations and errors in the expression in the variety of genes involved in cellular functioning.

Table 1: Statistical detail on Lung and Breast Cancer

	Lung cancer	**Breast Cancer**
Rank in Men/Women (M/W) or Women (W)	1 (M/W)	2 (W)
Age Susceptibility (major)	45-60 years	20-59 years
Relapse rate (within five years)	40%	11%
Survival rate (after 5 yrs)	4%	21%

Consequently, erroneous genes design faulty proteins with altered or under-regulated or dysregulated functioning thus; they cause harmful effects on growth, proliferation and other cellular activities. An awareness of the methodologies; how cells obtain and amalgamate extracellular signals, activating a flux of intracellular signals that manipulate cell development and metabolism, is necessary for the advancement a target-specific chemotherapy (Huang and Houghton 2003, Populo et al., 2012). Unfortunately by the time, cancer is detected in the patient the tumor metastasizes to other location in the patient's body and develops obstinate behavior towards treatment. Breast cancer can be further subdivided into: basal like breast cancer (BLBC) (includes triple negative breast cancer (TNBC) that ER negative, PR negative and HER-2 negative cases), second is a HER-2 enhanced type which involves over expression of HER-2 alone and luminal subtype having hormone receptor expression along with the presence or absence of HER-2 over expression.

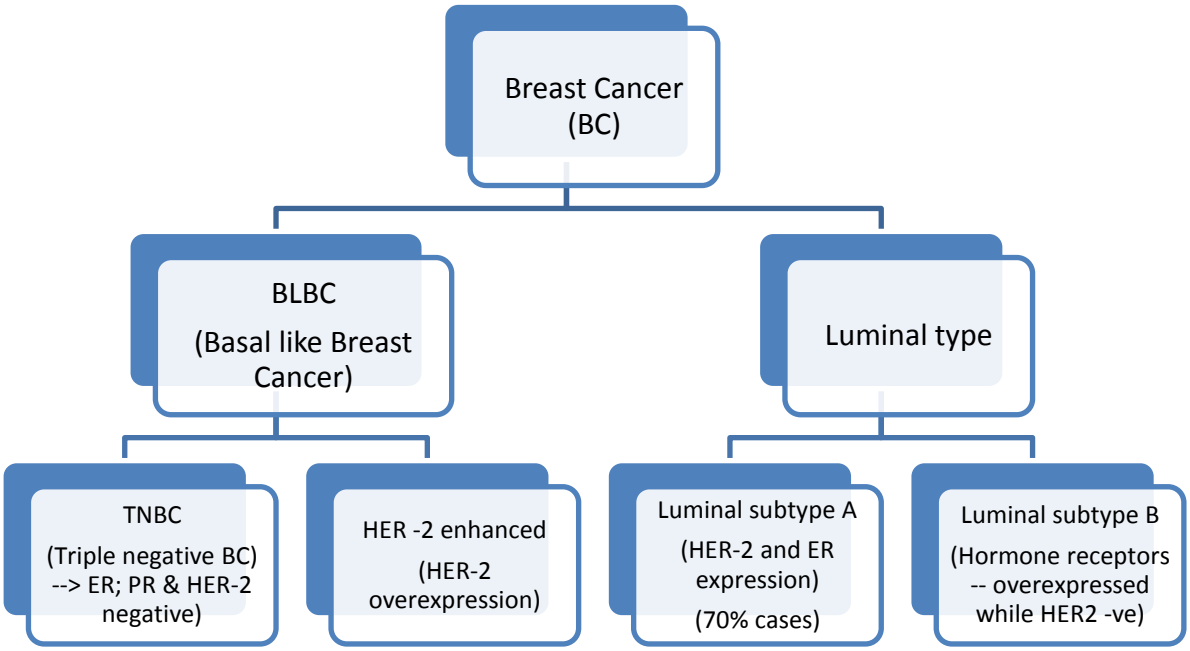


Figure 3: Breast Cancer Classification

Luminal subtype 'a' includes expression of both HER-2 and ER while luminal subtype 'b' consists of high hormone receptor expression with absence of HER-2 expression. NSCLC (non-small cell lung cancer) being the dominating one in case of lung cancer (LC) while in case of breast cancer (BC), the luminal breast cancer subtype 'a' consists of almost 70% cases among all the subtypes of breast cancer (Jafari et al., 2013, Paez et al. 2004).

Lung cancer (LC) is among one of those top four scoring cancers that are responsible for half of the cancer deaths in men and women (Mathers and Loncar 2006, Siegel et al. 2014) (Table 1). While as per Siegal et al., one half of the cancer cases projected for year 2014 in women will be lung, bronchus, breast, cervix and colon cancer, out of which Breast cancer (BC) consist of 29% of these all and thus being at first or second rank in case of occurrence among women (Koshki, Hajizadeh et al. 2013). As per a study reported, One-quarter of all deaths that happens due to cancer are because of LC only (Lee et al. 2011, Siegel et al. 2014, Wender et al. 2013). Majority of people that are aged beyond 60 are more prone to LC (Koshki et al. 2013, Toh and Lim 2007), while BC ranks first in case of women aged between 20 to 59 years.

Although from the last two decades cancer deaths rates has declined continuously but still not significant reduction in BC cases has been reported (Siegel et al. 2014, Toh

and Lim 2007). Similarly, due to enhanced diagnostic facilities and early detection and treatment initiatives breast cancer graph have shown a bit declination by 34%. Moreover there are chances of relapse of LC within five years following curative healing is 40% while surgery combined with standard chemotherapeutic treatments are not curative for metastatic BC patients as these have shown a survival rate of 7 to 8 % only in last 15 years (Gollob et al. 2006, Yu, Chen et al. 2008). Therefore more focus on the awareness, diagnosis and most importantly on therapeutic aspects is required (Yu, Chen et al. 2008). If we look at therapeutic aspects; lung cancer can be classified into small cell lung cancer (SLC) (20%) and non – small cell lung cancer (NSCLC) (80%). Particularly with Non-Small Cell Lung Cancer (NSCLC) is further sub classified into adenocarcinoma, squamous and large cell lung cancer (Brambilla and Gazdar 2009, Minna et al. 2004, Yu, Chen et al. 2008). However, majority of these consist of adenocarcinoma cases in which EGFR mutations takes place; thus, it can be easily observed that EGFR is the most potential target in smokers and non-smokers lung cancer patients (Lee et al. 2011, Minna et al. 2004). Further, observing the pathways and the enzymatic systems of our body, this is already reported that enzymatic system is divided into two major families' serine threonine kinase and tyrosine kinase (Faivre et al., 2006, Populo et al. 2012). Hence, if on one hand EGFR belongs to tyrosine

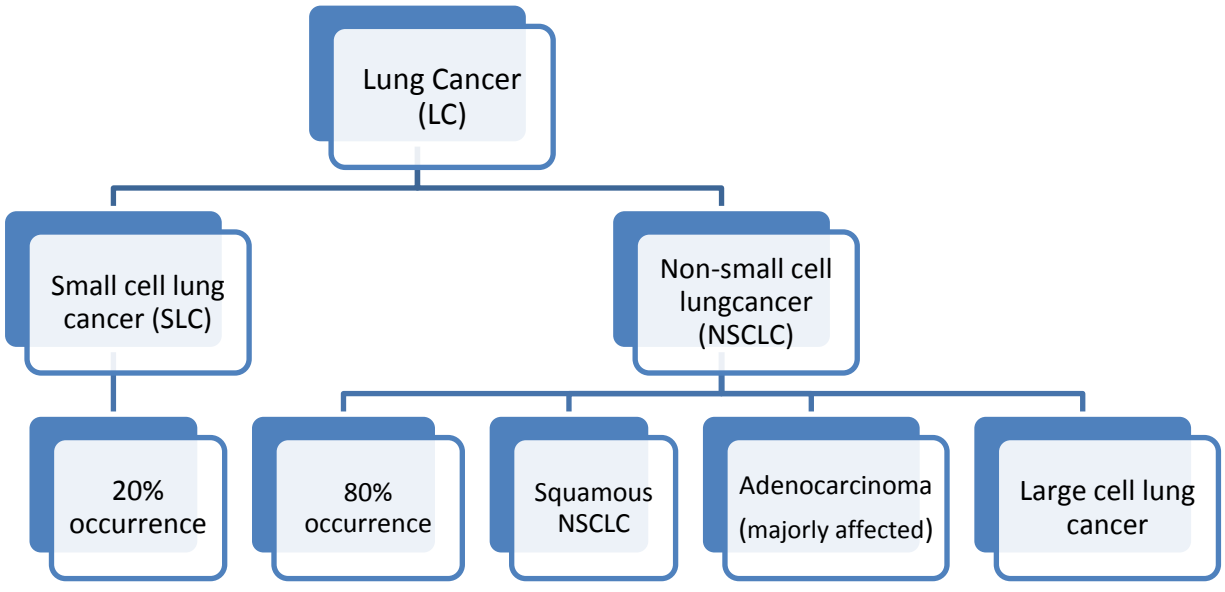


Figure 4: Lung Cancer Classification.

kinase family of receptors on the other hand, EGFR has its pathway linked to major components of serine/threonine kinase family, specifically PI3K/Akt pathway with downstream signaling to mTOR (Populo et al. 2012).These two components are activated during the signal transduction and downstream signaling. As per the studies conducted with these kinase families so far, it has been reported that mainly mutations take place in Tyrosine kinase. EGF Receptor undoubtedly has a crucial role to play in cancer, but also some additional activating somatic mutations take place in the EGFR signaling pathway. A considerable intensity of cross talk between receptors inside a signaling network and with other pathways has been regulating various functions of cell like its growth, survival, apoptosis, multiplication, etc. Like somatic mutation in PIK3CA (Phosphatidylinositol-4,5-bisphosphate 3-kinase, catalytic subunit alpha) gene that encodes for p110 catalytic subunit, PTEN, which has a tumor suppressor phosphatase gene lost its function of selective dephosphorylation of phosphatidylinositol 3, 4, 5-triphosphate (has been observed in a large range of cancers). It causes to enhance the activity of PI3K and more dependence on this pathway. These enzymatic systems function in intra and extracellular region through different signal transduction pathways (Scaltriti and Baselga 2006). In addition to all these studies, another noteworthy fact is; that lungs are one of the most prevalent sites for BC metastasis. And as per the studies performed so far, it is known that the tumor microenvironment plays a crucial role in metastasis and tumor progression. In case of BC metastasis also reasons were searched behind the prominent metastasis of breast to lungs. And in this regard studies reported that circulating cancer cells metastasis is based on their different demands for establishment to new locations according to the new cellular environment. Thus the information that, which receptors are needed to be targeted could be efficiently evaluated only if genes expression involved in these processes could be identified and correlated. Minn et al. in a study showed that genes that have the tendency to metastasize and relapse to lungs are; EREG (epiregulin, a member of epidermal growth factor receptor, MMP1 (collagenase 1 of matrix metalloproteineases), MMP2 (gelatinase A), S6K1 (S6 kinase1), 4EBP1 (Eukaryotic translation initiation factor 4E Binding Protein), eIF4E (Eukaryotic translation initiation factor 4E) and many others out of which above mentioned are the most prevailing one.

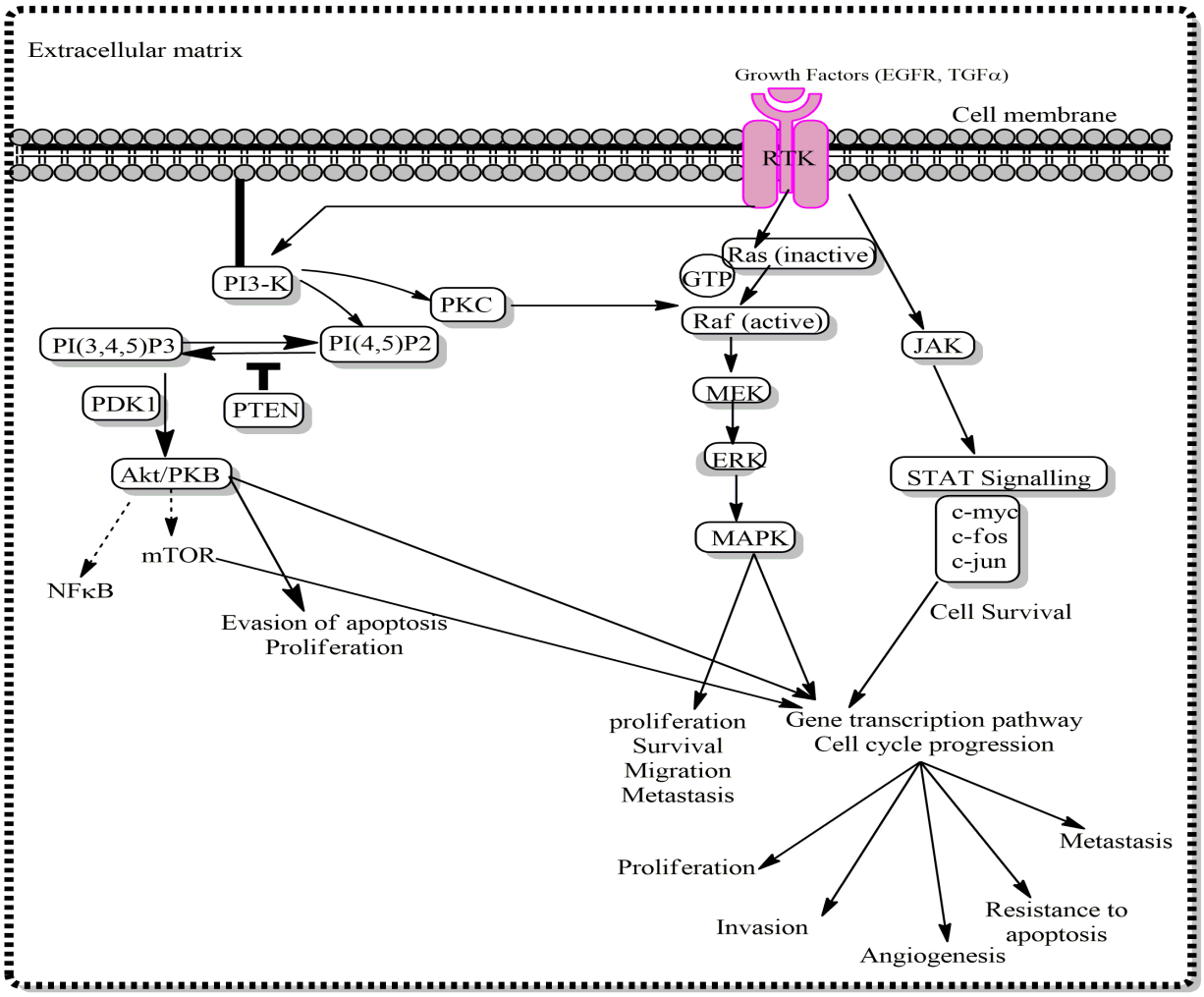


Figure 5: Crosstalk signalling pathway of EGFR kinase, Akt, mTOR and PI3Kinase receptors.

Another study has also shown the role of PTEN and PI3CK mutations in BC and lung cancer both (Populo et al. 2012) (Figure 5). Thus, pertaining to EREG gene which is precursor of EGFR protein receptor into consideration; further helps us in seeking a target from kinase family to overcome the breast metastasis as well as to target both lung and breast cancer simultaneously. Hence, apart from the hormonal receptor targets, EGFR, PI3K and Akt kinases along with mTOR that is activated by PI3K/Akt pathway; are other important targets for treatment of BC too (Faivre et al. 2006, Gollob et al. 2006, Minn et al. 2005).

Thus, to regulate any mutation or error in these pathways or proteins we need to target these pathways by affecting more than one of their components or enzymes. All these

components participate in cell growth, proliferation, survival and apoptosis. Therefore, for fear that if resistance is acquired against one of the components, the others will still be actively participating in fighting against cancer. Thus, these pathways have a crucial interlinking role in LC as well as BC. And if a multi-target approach is used against cancer, it may help in reducing the resistance acquisition, relapse occurrence, toxic effects and side effects problems.

1.3 Natural Products as Anticancer Resources

From ancient period, medicines and natural products have been related closely via uses of traditional medicines and natural poisons. Different studies were performed on traditional plants and medicines derived from them by performing clinical, pharmacological and chemical studies. Drugs like aspirin, digitoxin, morphine, quinine and pilocarpine (Butler 2004) are the good example in this context. As per study, approximately more than 25% of modern medicines are straight or obliquely obtained from natural sources. The past have been evident for the fact that remedial flora of South Asian region are measured among the major resource of numerous pharmacologically important compounds that are usually taken as home remedies in curing or treating various types of diseases ranging from common cold to life threatening cancer (Patel 2012). Among all the therapies implemented so far to treat cancer, involves use of synthetic, semi-synthetic, synthetic- natural product mimic and naturally derived products (Newman & Cragg, 2012). From past few years, it has been observed that patients of cancer are also attracted more towards natural therapies of complementary and alternative medicines. For example, Vincristine, Vinblastine, Topotecan, Irinotecan, Etoposide and Paclitaxel are all derived from natural sources. These are the molecules that are incorporated in cancer treatments, and molecules derived from microbial sources like Dactinomycin, Bleomycin and Doxorubicin are also being used as anticancer agents (Schwartsmann et al., 2001). Some are under clinical trials like, Elsamicin, Chartreusin the compounds binding to DNA, and inhibiting topoisomerase II activity also, Brostallicin DNA minor groove binding and Geldanamycin, a polyketide natural product is under investigation for inhibition of protein chaperone heat shock protein (HSP) 90. Some more examples are like,

Spicamycin, Becatecarin, edotecarin, midostaurin etc.; (Kinghorn et al., 2006). Promising possibilities, positive results with lesser side effects and good physiological tolerance by patients at optimal dosage regimens are being offered by naturally derived experimental molecules, for analyzing totally different new drug classes of anticancer agents, and also many novel and promisingly acceptable mechanisms of action (da Rocha et al., 2001). On the basis of earlier positive outcomes of past attempts and research work being carried out on natural products still now, here we have also focused our studies in the current report on one of the natural golden herb, which are expected to give the promising results in searching out and preparing an anticancer agent that may bring cure for cancer, on the basis of studies performed on it so far. This golden herb is Cassia fistula. It is famous by the name “Aragvadha” meaning “disease killer” in Ayurveda or Amaltash in Hindi.

Through this information and significance of medicinal plants in the development of therapeutic agents, *Cassia fistula* was selected for study of the following objectives.

The following are the objectives of the present dissertation work:

1. In vitro anti-proliferative studies of different extracts/fractions from Cassia fistula against different cancer cell lines
2. To study the molecular interactions of reported secondary metabolites from Cassia fistula with different targets like EGFR, Akt, PI3K, mTOR through in silico approach.

CHAPTER – 2

REVIEW OF LITERATURE

Chapter – 2

Review of literature

2.1 Introduction

From ancient period medicines and natural products have been related closely via uses of traditional medicines and natural poisons. Different studies were performed on traditional plants and medicines derived from them by performing clinical, pharmacological and chemical studies for example on aspirin, digitoxin, morphine, quinine and pilocarpine (Butler, 2004). As per a study approximately more than 25% of modern medicines are directly or indirectly derived from natural sources. History shows that medicinal plants of India are considered as major source of several pharmacologically important compounds that are commonly used as home remedies against various types of diseases that can range from common cold to life threatening Cancer (Patel, 2012). Indian laburnum, golden shower, pudding pipe and known by many other names the plant, Cassia fistula Linn. (Leguminoseae) (Figure 6) is also known as “rajavrska” in Sanskrit and can be considered as a great representative of natural healer plants.

Figure 6: Leaves and flowers of *Cassia fistula* L. (Courtesy: Photograph taken from the campus of Central University of Punjab, Bhatinda)

As per Ayurveda, it is called as “Aragvadha” meaning “disease killer” plant (Lim, 2012; Pole, 2006). It is also known as Amaltas; is a deciduous tree and grows throughout the greater parts of India. It is native to India and has become extensively diffused in various countries including Mauritius, Sri Lanka, Amazon, Malaysia, South Africa, Mexico, China, West Indies, East Africa and Brazil as an ornamental tree and known for its beautiful bunches of yellow flowers. It is used as an ornamental plant also, because of its beautiful appearance and various medicinal uses (Neelam et al., 2011; Shailajan et al., 2013). It belongs to family Leguminoseae, within subfamily Caesalpiniodeae. Ayurveda represents its energetics as, sweet in taste, with energy in cold form, post digestive effects as sweet and quality being heavy. C.fistula has effects on all three doshas – vata, pitta, kapha (it pacifies vata and purges pitta and kapha). It alleviates vayu and pitta dosha. As per Ayurveda, it loses its properties on heating (Pole, 2006). Various uses of amaltash as a whole herb, known to date are many; every part of this herb has its unique importance (Danish et al., 2011).

2.2 Traditional uses of *Cassia fistula:*

Roots of amaltash are considered as remedy for fever, heart diseases, retained excretions and biliousness, rheumatic condition, haemorrhages, wounds, ulcers, boils and various skin diseases chest pain, joint pain, migraine as well as blood dysentery, tonic, astringent, febrifuge and strong purgative. Root’s alcoholic extract can be used for lowered the blood sugar level up to 30 % and in black water fever also. Amaltash mixed with goat milk are given in little doses regularly for the treatment of breast diseases (Neelam et al., 2011).

Leaves are considered as laxative, external emollient, and poultice is used for chilblains, in insect bites, swelling, rheumatism and facial paralysis. These possess anti periodic properties, are being used in jaundice, piles, rheumatism ulcers and also externally skin eruptions, wounds, ulcers, prurigo, pruritis, eczema. Leaves juice is utilised as dressing for ringworm, relieving irritation and relieving dropsical inflammation, in jaundice treatment (Danish et al., 2011).

Seeds used to treat dermal diseases, abdominal pain, chronic and acute constipation, acidity and leprosy. It has been reported to have cooling and anti-pyretic properties. It has mild sweet taste and possesses laxative, carminative properties. Seeds possess hypoglycaemic activity (Neelam et al., 2011). These are mild emetic, cathartic, and are helpful in treating biliousness, skin disease and swollen throat, these when dried, have marked hypoglycaemic activity (Danish et al., 2011).

Flowers are used to treat dermal infections, gastrointestinal pain and leprosy. They act as antipyretic. They have wound healing characteristic, astringent action and purgative action. There decoction treats stomach troubles, relieve cough, inhibit ovarian function in rats anti-fertility (Danish et al., 2011; Neelam et al., 2011).

Fruits are considered relieving as cathartic, in asthma, diabetes, fever, abortifacient, demulcent, lessening inflammation and heat of the body, chest problems, throat troubles, hepatic problems, ophthalmic problems, gripping and in snake bite, colic, choleresis, urinary infections (Danish et al., 2011).

Pulps and pods are safe purgative for children and pregnant women. These are used in hepatic disorders, biliousness, as a tonic, also applied in gout, rheumatism, in malaria and black water fever also (Neelam et al., 2011). It treats blood - poisoning, anthrax, dysentery, leprosy, diabetes and relieves gastrointestinal problems. They relieves cough, reduce throat inflammation when heated pods applied on neck. They act as analgesic, abortifacient, potent clastogens (Danish et al., 2011; Neelam et al., 2011).

2.3 Pharmacological Activities (Figure 7)

2.3.1 CNS activity

The methanol extract (ME) of the C. fistula seeds noticeably enhanced the sedative actions of sodium pentobarbitone, diazepam, meprobamate, and chlorpromazine. It is reported to reduce pain significantly when induced by morphine and pethidine in a dose dependent manner (Danish et al., 2011; Mazumder et al., 1998).

2.3.2 Antioxidant

Antioxidant activities of reproductive parts of C. fistula have been found to be greater than those of the vegetative organs, where pods have highest total phenolic, proanthocyanidin, flavanoid contents and antioxidant potentials. The total phenolic, proanthocyanidin and flavanol content add up synergistically to show anti-oxidant activity. The pronounced antioxidant property observed in the reproductive organs can mainly be attributed to the high levels of proanthocyanidins (flavanol derivatives) comprising mainly catechins, oligomeric and polymeric proanthocyanidins (Bhalodia et al., 2011; Luximon-Ramma et al., 2002). Catechins, oligomeric proanthocyanidins, emodin, aloe-emodin, have free radical scavenging and antilipoperoxidant activities in various antioxidant assay systems (Bhatnagar et al., 2010).

Catechins and kaempferol found in C. fistula have good antioxidant index when compared with the antioxidant index of reference compounds (Luximon-Ramma et al., 2002). The production of hydroxyl radicals are accelerated by anthraquinone, alizarin, chrysophanol and rhein (Chuang et al., 2000). The antioxidant activity power was in the decreasing order of stem bark, leaves, flowers, pulp when correlated with their total polyphenolic content of the extracts (Siddhuraju et al., 2002). In one of the study on antioxidant, flowers being most potent in action (75.15% in 1.0µg/ml) with DPPH model assay (Nehru et al., 2008). As per another study, the antioxidant activities follow the order: pod > flower > stem bark > leaves > pulp with direct relation to the content of total phenols and flavanoids in its extract (Rizvi et al., 2009).

2.3.3 Anti-fertility and Abortifacient activity

Oral administration of aqueous extract of seeds of C. fistula to mated female rats from day 1-5 of pregnancy at the doses of 100 and 200 mg/kg body weight resulted in 57.14% and 71.43% prevention of pregnancy, respectively, whereas 100% pregnancy inhibition was noted at 500 mg/kg (Bahorun et al., 2004). Orally administered seed extracts of C. fistula prevented pregnancy and decreased uterine implantation (Rizvi et al., 2009; Yadav & Jain, 1999). A study suggests that the petroleum ether extract of C. fistula seeds possesses contraception effects by virtue of anti-implantation activity (Yadav & Jain, 2009). A study conducted recently, indicate that the extract possess

anti-estrogenic property which may be responsible, at least partly, for the anti-contraceptive effect (Malpani et al., 2010).

2.3.4 Anti-diarrheal and purgative

The plant species has potential antidiarrheal components (Kuo et al., 2002). It is also a purgative due to the wax aloin and a tonic (Bahorun et al., 2004). Roots of C.fistula is reported to have a strong purgative action, while fruit pulp proved to possess the laxative action (Ved et al., 2010).

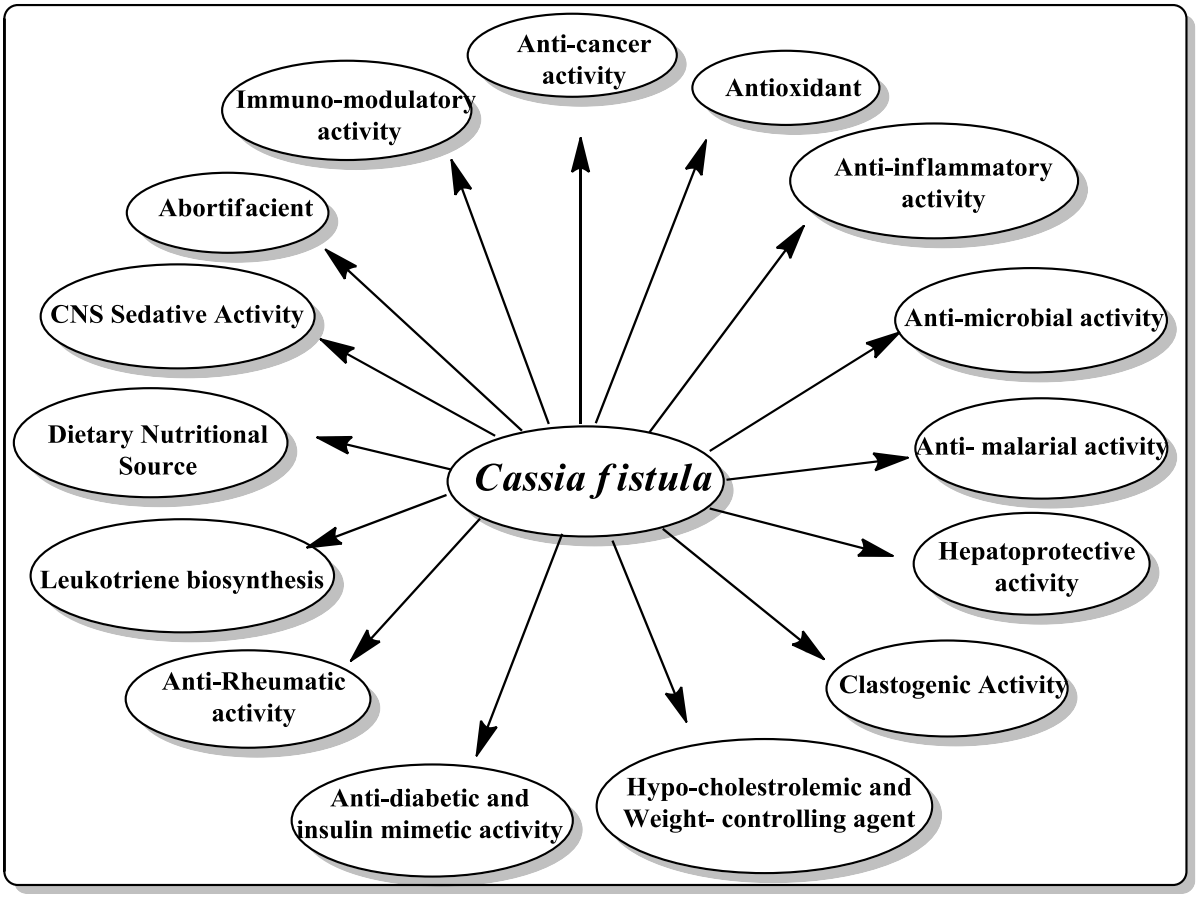


Figure 7: Reported pharmacological activities of *Cassia fistula* Linn. .

2.3.5 Antibacterial/ Antimicrobial activity

C. fistula seeds with lectins found active against 14 pathogenic bacteria, including strong action against Bacillus megaterium, Streptococcus *β*-haemolyticus and Shigella boydii (Sayeed et al, 2003). Antibacterial activity against a wide spectrum of bacteria namely Escherichia Coli, Bacillus mycides, Bacillus subtilis, Mycobacterium smegmatis, Klebsiella aerogenes, Pseudomonas aerogenes and Proteus vulgaris

(Bahorun et al., 2004; Ignacimuthu et al,, 1998; Rizvi et al., 2009). Antibacterial activity of petroleum ether, methanolic and ethyl acetate extract of seeds, stem bark and leaves of C. fistula have been reported against B. subtilis, B. mega-terium, S. haemolyticus, S. aurus, S. lutea, S. sonnei, E. coli, K. sps, S. shiga, S. boydii, S. flexnerae, S. dysen-teriae, S. typhi and P. aeruginosa. Its pods and stem bark are highly active (Mahida & Mohan, 2006; Raghavan et al., 2006; Sohel & Yeasmin, 2004). C.fistula leaves have shown more potent zone of inhibition for S. aureus and P. aeruginosa (Senthil Kumar et al., 2006). Hexane, chloroform, ethyl acetate, methanol and water extract of flowers have shown antibacterial activity against S. aureus, S. epidermidis, B. subtilis, E. faecalis and P. aeruginisa (Duraipandiyan & Ignacimuthu, 2007; Rizvi et al., 2009). The pod and stem bark extracts of C. fistula were found active against Ranikhet disease virus (RDV) and Vaccinia virus and fruit extract gave activity against Foot and Mouth Disease Virus (FMDV) (Rizvi et al., 2009). The alcoholic extract of leaves of C. fistula antimicrobial activity against S. aureus, P. aeruginosa, E. coli and Group A. streptococcus, some common human pathogens is reported. The studies have shown that it has bacteriostatic property instead of bactericidal activity. This is due to presence of anthraquinones containing phenolic groups in high concentration (Vasudevan et al., 2009). C. fistula seeds have shown antimicrobial activity against Candida albicans and Artemia salina (Lachumy et al., 2010).

2.3.6 Anti-inflammatory action

C. fistula's significant anti-inflammatory effect has been reported in both acute and chronic models. The presence of flavanoids may be responsible for the anti inflammatory effects (Abbas et al., 2008; Yadava & Verma, 2003). The Lipid peroxidation (LPO) implies for the pathogenesis of various diseases including arthritis. It is well established that bio-enzymes are very much susceptible to LPO, which is considered to be the starting point of many toxic as well as degenerative processes. The extracts of C.fistula exhibited protection against lipid peroxidation induced by CCl_4. There was a dose dependant manner inhibition from extracts of C.fistula of $FeSO_4$ induced lipid peroxidation. This prevention could be caused by the inhibition of formation of ferryl perferryl complex (Bahorun et al., 2004; Ilavarasan et al., 2005). The

administration of ethanolic extract of C.fistula at higher doses suggested that it possess anti inflammatory action comparable to those of diclofenac and indomethacin (Gobianand et al., 2010). Its anti-inflammatory activity was found comparable to that of phenylbutazone, when tested on carrageenan, histamine and dextran-induced paw oedema assay in rats (Tejendra Bhakta et al., 2000; Patel, 2012).

2.3.7 Anti-ulcer

It treats many other intestinal disorders like healing ulcers (Bahorun et al., 2004; Ghosh & Biswas, 1973; Kirtikar, Basu, & Blatter, 1975), and can used to treat ringworm infection (Kirtikar et al., 1975; Rizvi et al., 2009). Ethanol leaf extract (ELE) of C. fistula (Leguminoseae) was assessed for antiulcer activity against pylorus ligation-induced gastric ulcer; its activity was evidenced by the considerable attenuation of gastric volume, pH, free acidity, and total acidity in the gastric juice of pyloric-ligated rats in a dose-dependent manner. The protective effect was expected to be due to strengthening of the mucosal defense mechanism. ELE pre-treatment noticeably attenuated the fall in status of sialic acid and fucose accompanied by an increase in hexose, hexosamine, total non-amino polysaccharide, total carbohydrate, and C:P ratio in the gastric juice of pylorus-ligated rats and this effect could be due to protection of the mucosal barrier system. ELE pre-treatment significantly prevented the increase in LPO and SOD, in the gastric juice of pyloric-ligated rats. The protective ability of ELE against pylorus ligation-induced gastric ulcer could be attributed to its free radical scavenging and antioxidant properties. The antiulcer activity of ELE could be attributed to (i) a decrease in gastric acid secretion, (ii) protection of the mucosal barrier and restoration of mucosal secretions, (iii) inhibition of free radical generation or prevention of lipid peroxidation, and (iv) free radical scavenging or antioxidant properties (Karthikeyan & Gobianand, 2010).

2.3.8 Antipyretic and Analgesic

It showed antipyretic and analgesic effect (Bahorun et al., 2004). Ethanolic extract of C. fistula possesses antipyretic activity attributed to the presence of alkaloids, terpenes and glycosides (Gobianand et al., 2010). The methanolic extract of buds of C.fistula showed significant activity in rat's models at doses of 200 and 400 mg/kg. At both

dosage levels dose dependent lowering of body temperature took place up to 4 h (Bhakta et al., 2001; Patel, 2012).

2.3.9 Hepatoprotective

C. fistula leaf extract in n-heptane was found useful in treating liver troubles, as a hepatoprotective (Bahorun et al., 2004; Bhakta et al., 1999). The aqueous extract of C.fistula bark also showed hepatoprotective activity against CCl_4 induced liver toxicity in rats. It minimizes the hepatocytic damage, attenuates the initiation and progression of lipid peroxidation (Parthasarathy & Prasanth, 2009). It has potential ability to be a hepatoprotective agent, even when compared to silymarin drug as reference and fruit pulp showed good hepatoprotective action with particular extent (Das et al., 2008). Leaves extract significantly reduced SGOT, SGPT, bilirubin and alkaline phosphatase levels in paracetamol induced hepatotoxicity (Bhakta et al., 2001; Rizvi et al., 2009). Studies have suggested that high dose of ethanolic extract of leaves (500 mg/kg) body weight reported to have hepato-protection against Isoniazid/Rifampicin induced hepatitis in rats (Jehangir et al., 2010).

2.3.10 Anti-Periodic and Anti-Rheumatic

C. fistula extract is used as an anti-periodic agent and in the treatment of rheumatism (Bahorun et al., 2004; Kirtikar et al., 1975). A study suggests that the plant has emenagogue property (Alexandros, 2007). It treats rheumatic disorders, DNA damage and ageing (Ilavarasan et al., 2005; Rizvi et al., 2009). The fruit pulp possesses favourable activity in arthritis (Ved et al., 2010).

2.3.11 Hypo-cholestrolemic and Weight Controlling Actions

Hypo-cholesterolaemic action is partially due to their fibre and mucilage content that significantly reduced blood and liver total lipids (Bahorun et al., 2004; El-Saadany et al.,1991). Blood, liver, kidneys, and spleen and heart total cholesterol was significantly reduced. A marked progress in the correction of lipid metabolism occurred. C. fistula induced a significant decrease in high activities of serum GOT, GPT, alkaline and acid phosphatase (El-Saadany et al., 1991). Bark has shown antihyperlipidaemic effect by improving lipid profile with β-sitosterol present in stem bark. It reduced total cholesterol with triglycerides and LDL-cholesterol levels along with increasing HDL cholesterol content (Daisy et al., 2008). The antioxidant property can in turn be linked with heart

disease (Deshpande et al., 1996; Willett et al., 1993). Administration of its legume extract along with cholesterol inhibited the elevation in serum total, LDL-cholesterol, triglycerides and phospholipid at the doses 100, 250 and 500 mg/kg b.wt./day in a dose dependent manner (Gupta & Jain, 2009). It possesses tannins which are responsible for weight reducing potential of the plant and studies in this context were performed on 3 weeks old mice. Tannins are reportedly involved in growth regulation and lipase inhibiting action in mice that helps in reducing body fat content (Chichioco-Hernandez et al., 2011).

2.3.12 Anti-asthmatic, Anti-allergic, Cardio-Vascular Disease modulator

Studies have shown its inhibitory effect on leukotriene biosynthesis. C. fistula inhibited the 5-lipoxygenase catalysed formation of leukotriene B4 in bovine polymorphonuclear leukocytes (IC50 value of 38µg/ml) (Danish et al., 2011; KC & Müller, 1998). This represents the importance of C.fistula in asthma, allergic diseases like rhinitis and anaphylaxis. Also it has been observed in last few years that leukotirene modifiers can be useful in treating risk of heart diseases like atherosclerosis, myocardial infarction, aortic aneurysm etc (Funk, 2005).

2.3.13 Anti-malarial activity

C. fistula is reported to have antimalarial activity (Alexandros, 2007). The significant repellence against Aedes aegypti has been reported from crude extract of leaves. Results explained the potential larvicidal, ovicidal and repellent activity of C. fistula against chikungunya vector mosquito, Aedes aegypti (Govindarajan, 2009).

2.3.14 Anti-hyperglycemic and insulin mimetic effect

When tested in reference to glibenclamide and other thiazolidindiones its bark successfully reduced the increased blood glucose level in streptozocin induced diabetic rats. Antioxidant and polyphenolic content are expected to be responsible for hypoglycaemic response (Nirmala et al., 2008). A study suggests that catechin is having hypo-glycaemic, glucose oxidizing and insulin mimetic activities, and thus it can be considered as promising future anti-diabetic agent (Daisy et al., 2010). A recent study conducted with the help of ligand based drug design and based on earlier in-vitro studies proved that catechin is capable of activating insulin receptor and Peroxisome proliferator-activated receptor gamma, confirming the hypoglycaemic effect of catechin

(Pitchai & Manikkam, 2012). A study suggests that dietary fibres present in seeds of C.fistula can also help in reducing postprandial glucose levels (Mishra & Jha, 2011). Because of richness in protiens and minerals seeds flour can be incorporated in daily meals (Akinyede & Amoo, 2009). Seeds are rich source of calcium also, making it more beneficial (Rizvi et al., 2009).

2.3.15 Anti-Parasitic / Anti-leishmanial

Dichloromethane extract of plant showed Anti-leishmanial activity. An active isoflavone compound Biochanin A isolated from fruit was found to be active specifically against trypanosome-cruzi with an EC_{50} value of 18.32µg/ml and 2.4 fold more active than benznidazole. Biochanin A showed promising opportunities to design a new therapeutic drug against Chagas' disease (Sartorelli et al., 2009).

2.3.16 Humoral immunity and immuno-modulation

C.fastula has immuno-modulatory action and hence found to enhance humoral immunity. Along with this, they have also shown synergistic effect on the antimicrobial action of amoxicillin (Faizi et al., 2008). Leaf extract of C.fistula have anti-tussive effect (Bahorun et al., 2004). It has shown anti-cough effects in mice with reflux induced cough (Bhakta,et al., 1998; Rizvi et al., 2009)

2.3.17 Toxicity low level

Pods of C. fistula showed no pathological effects on liver, kidney and testis of rats, and are found to have very low toxicity levels at almost 50% lethal dose (6600mg/kg/bw) (Akanmu et al., 2004; Rizvi et al., 2009).

2.3.18 Anti-fungal

A study suggests that fruit pulp and seed extract possess anticandidal activity against Candida albicans, Candida glabrata and Candida tropicalis (Rizvi et al., 2011). In other study, antifungal activity of rhein (1, 8-dihydroxyanthraquinone- 3carboxylic acid) from flowers has been reported. Rhein inhibited the growth of many fungi such as Trichophyton mentagrophytes (MIC 31.25µg/ml), Trichophyton simii (MIC 125 µg/ml), Trichophyton rubrum (MIC 62.5 µg/ml) and Epidermophyton floccosum (MIC 31.25 µg/ml) (Duraipandiyan & Ignacimuthu, 2010; Ramakrishna & Indra, 1997; Rizvi et al., 2009).

2.3.19 Larvicidal and ovicidal

A study conducted in 2008, showed that the leaf extract is promising as a larvicidal and ovicidal agent against C. Quinquefasciatus as well as A. Stephensi (Govindarajan et al., 2008). The ovicidal effect of leaf extracts (at 0.5, 1.0 and 2.0%, topically applied) was evaluated on the viability and hatching of eggs of D. koenigii. Application of leaf extracts inhibited hatching of the eggs as concentration of the extract was increased resulted in increased non-viability of 3-day-old eggs (Danish et al., 2011).

2.3.20 Clastogenic activity

Anthraquinone glycosides of C. fistula for their ability to induce a clastogenic effect have demonstrated by Mukhopadyay et al 1998. Oral exposure to anthraquinones doses and their equivalent amount in leaf and pod extracts did not induce significant numbers of chromosomal aberrations or aberrant cells. Results indicate that anthraquinone sennoside B and rhein are weakly genotoxic. Leaves and pods extracts which also possess same phyto-constituents were very weak clastogens. Thus, these phytolaxatives do not behave as potent clastogens and pods or leaves can be used as an alternative source of sennosides (Danish et al., 2011).

2.3.21 Antitumor activity

The studies performed on antitumor activity in Erlich ascites carcinoma showed positive results. Cytological studies revealed a reduction in mitotic activity alongwith appearance of membrane blebbing and intracytoplasmic vacuoles in the treated cancer cells. Improvement in the hematological parameters following ME treatment, like haemoglobin content, red blood cell count and bone marrow cell count of the tumour bearing mice have also been observed (Bahorun et al., 2004; Gupta et al., 2000). Anticancer activity of leaves and fruit parts of the plant have been reported (Rahmatullah et al., 2009). In a study performed by Ved, Bharate et al., 2010 ethanolic extracts of C. fistula along with few other plants were prepared to study through bench top assay; the cytotoxicity on Brine shrimp solution. Extracts were prepared using soxhlet extraction procedure; involving packing of plant materials of approx. 25g. The stock solutions prepared at concentrations of 10mg/ml. Fruit extract of C. fistula was found to possess cytotoxic activity with percentage lethality up to 73±22 at dose concentration of 1000µg/ml solution (Ved et al., 2010). A recent study performed on

antitumor activity of ethyl acetate extract of flowers and rhein against colon cancer cell lines suggested that C.fistula have anticancer potential. The chemopreventive efficacy of bark extracts of C. fistula in 7, 12-dimethyl benz(a)anthracene (DMBA) induced hamster buccal pouch carcinogenesis. Oral administration of bark extract to DMBA painted animals completely prevented the formation of oral squamous cell carcinoma. Bark extract also restored the status of lipid peroxidation by-products, antioxidants and detoxification enzymes in DMBA painted animals. The chemo-preventive potential may also be due to its anti-lipid-peroxidative, anti-oxidative and modulation of detoxification agents during DMBA induced oral carcinogenesis (Danish et al., 2011; Rizvi et al., 2009).

2.3.22 Skin whitening efficacy

Methanolic extract of pods effectively diminshed the harmful effects of solar damage and hyperpigmentation/ melasma. It was found to be due to presence of various polyphenols, alkaloids, fatty acids, flavanoids like catechins, epicatechin, epigallocatechins, quercetin etc. that showed anti-tyrosinase activity. There activity against tyrosinase resulted in reduction in melasma (anti-melasmic effects) (Khan et al., 2013).

2.4 Phytochemistry of *Cassia fistula* L.

The extensive research work has been performed on primary and secondary metabolites of C. fistula. Different combination of chemical constituents present in C. fistula that make it as Aragvadha or disease killer plant. It contains good quantity of carbohydrates, amino acids, fatty acids, various micronutrients and proteins as primary metabolites. As far as secondary metabolites are concerned amaltash contains a combination of glycosides, flavonoids, essential oils, terpenoids, proanthocyanidins and polyphenols. These are the moieties which are proven to carry a variety of pharmacological activities like, antimicrobial, anti-inflammatory, antifungal, antitumor, antioxidant and anti-diabetic etc. The vegetative and reproductive parts contains catechin, oligomeric proanthocyanidins (flavanol derivatives), flavanoids, kaempferol (Luximon-Ramma et al., 2002). A study performed in 1989 on C. fistula suggested that anthraquinone are present in bound form not in unbound form (Rasul et al., 1989).

Leaves are found to possess (-)-epiafzelechin (**1**), its 3-O-glucoside (**2**), (-)-epicatechin (**3**), procyanidin B-2 (**4**), seven biflavanoids and two triflavanoids out of these three biflavanoids and two triflavanoid are reported as proanthocyanidins each having (-)-epiafzelechin (**1**) unit in their moiety. The seven reported biflavanoids are Epiafzelechin-(4β-8)-epiafzelechin **(5)**, epiafzelechin-(4β-8)-epicatechin **(6)**, epicatechin-(4β-8)-epiafzelechin **(7)**, (2S)-7,4'-dihydroxyflavan-(4β-8)-epiafzelechin **(8)** and (2S)-7,4'-dihydroxyflavan-(4α-8)-epiafzelechin **(9)** (2S)-7,4'-dihydroxyflavan-(4β-6)-epiafzelechin **(10)**, (2S)-7,4'-dihydroxyflavan-(4α-6)-epiafzelechin **(11)**. Two tri flavanoids are epiafzelechin-(4β-8)-epiafzelechin-(4β-8) –epiafzelechin **(12)** and (2S)-7, 4'-dihydroxyflavan-(4β-8)-epiafzelechin-(4β-8)-epiafzelechin **(13)** are the proanthocyanidins found in the leaves (Ezaki et al., 1985). The total phenolic content possessed by leaves is 19% as per a study conducted at 2002 (Siddhuraju et al., 2002). These have not been used for laxative action, but are reported to contain sennosides B **(14)**, chrysophanic acid **(15)**, physcion **(16)**, citreorosein **(17)** and a major constituent rhein. The total anthraquinone glycosides content in was found to be 1.52% as dry weight (Gritsanapan & Nualkaew, 2008). Oxalic acids (**18**), tannins, oxyanthraquinones, anthraquinones are also present (Bhalerao & Kelkar, 2012). The cuticular wax of leaves contain hextriacontanoic, triacontanoic, nonacosanoic and heptacosanoic acids (Danish et al., 2011). The essential oils extracted from leaves at the yield of (0.012%) with relative concentration of oils as eugenol (25.0%), (E)-phytol (21.5%), camphor (13.5%), limonene (11.0%), Salicyl alcohol (10.4%), linalool (9.9%), and 4-hydroxybenzenemethanol (8.7%) (Satyal et al., 2013).

The Stem Bark contains anthraquinones, proanthocyanidin, xanthones **(19)**, and flavonols with highest percent of 69.4%, of polyphenolics among vegetative parts of the plant (Siddhuraju et al., 2002). A bioactive flavone glycoside 5,3',4'-tri-hydroxy-6-methoxy-7-O-alpha-L-rhamnopyranosyl-(1 --> 2)-O-beta-D-galactopyranoside with antimicrobial activity was reported in 2003 by yadava and verma (Yadava & Verma, 2003)**.** The C.fistula also contains alkaloids and flavanoids as per a report (Bahorun et al., 2004; Duraipandiyan & Ignacimuthu, 2007). Recently two new furano-flavones determined that are named fistula-flavones A and B. Structural of the compounds were

found to be 3,5- dihydroxy-4'-methoxy-7,8-(2"-ethyl furan)-flavone **(20)** that is fistula A and structure of fistula-flavone B 3-methoxy-4'-5dihydroxy-7,8-[2"-(2-hydroxyethyl) furan]-flavone **(21)** (Gao et al., 2013).

In the Seeds of Cassia fistula a study reported, eight compounds, 5-(2-hydroxyphenoxymethyl)furfural **(22)**, (2′S)-7-hydroxy-5-hydroxymethyl-2-(2′-hydroxypropyl)chromone **(23)**, benzyl-2-hydroxy-3,6-dimethoxybenzoate **(24)** and benzyl 2â-O-D-gluco-pyranosyl-3,6-dimethoxybenzoate **(25)**, together with compounds, 5-hydroxymethylfurfural **(26)**, (2′S)-7-hydroxy-2-(2′-hydroxypropyl)-5-methylchromone **(27)**, and two oxyanthraquinones, chrysophanol **(28)** and chrysophanein **(29)**, were isolated and identified (Kuo et al., 2002).
Seeds were characterized as a new bioactive flavones glycoside 5,3',4'-tri-hydroxy-6-methoxy-7-O-α-L-rhamnopyranosyl-(1-2)-O-β-D-galactopyranoside, 5,7,3',4'-tetrahydroxy-6-methoxyflavone (Yadava & Verma, 2003). Seeds are characterized with presence of galactomannan in combination with 8 different types of sugar moieties (Bhalerao & Kelkar, 2012). It contains fatty acids like, glycerides with linoleic acids, oleic acids, stearic acids, and palmitic acids as major one, while, traces of caprylic acid and myristic acid. Proteins like globulin, albumins are present in it. It contains galactomannans and cephalins and lecithins phospholipids as primary metabolites. The seed oil contains cyclopropenoid fatty acids, viz, vernolic, malvalic and stetculic acids (Danish et al., 2011). The oil from seeds when fractionated by silicic acid column chromatography, it was found that mono-, di-, and tri-glycerides are present. The triglycerides varied from 89.16% to 91.01%, di-glycerides from 2.51% to 3.32% and mono-glycerides from 0.91% to 0.98% depending on the areas from which the seeds were collected. Lipids were fractionated into three major lipid groups neutral lipids, glycolipids and phospholipids were carried out by silicic acid column chromatography. The neutral lipids were accounted for over 89.80% of the total weight of the lipid employed. Saturated and unsaturated fatty acids present in the oil were separated and varied from 23.79% to 28.20% and 63.28% to 66.71% respectively depending on the areas. The major fatty acids analysed by GLC, found in the oil were linoleic acid (42.42%), oleic acid (29.62%), stearic acid (14.33%) and palmitic acid (11.41%). In

addition to the above, caprylic acid (0.76%) and myristic acid (1.44%) were also present in minor amounts (Danish et al., 2011). Three lectins, i.e. CSL-1, CSL-2 and CSL-3, purified from the Cassia fistula seeds and were tested for their antibacterial activities against different pathogenic bacteria (Sayeed, 2003).

The Heartwood contains Fistucacidin **(30)**, an optically inactive leucoanthocyanidin-5,4'-dihydroxyflavan-3,4-diol **(31)** ,(3,4,7,8,4' pentahydroxyflavan) was extracted from the heartwood (Bahorun et al., 2004; Duraipandiyan & Ignacimuthu, 2007; Padmanabha Rao & Venkateswarlu, 1965).

The Flowers of Cassia fistula are reported to have kaempferol **(32)** and a proanthocyanidin whose structure has been established as a leucopelargonidin tetramer having a free glycol in the acetone extract of the flower in 1972 (Bahorun et al., 2004). These also have polyphenols in good concentration of around 6.52**%** (Siddhuraju et al., 2002). Aurantiamide acetate (0.011), β-sitosterol (0.006) and its β-D -glucoside (0.02%) has been isolated from flowers (Danish et al., 2011). When flowers of C.fistula were examined; the chemical compositions of the flower and leaf essential oil by GC and GC/MS. Forty-four compounds were identified representing 92.6% and 90.7% of the flower and leaf oil, respectively. The main components of the flower oil were (E)-nerolidol (38.0%), and 2- hexadecanone (17.0%), while the leaf oil consisted mainly of phytol (16.1%) (Danish et al., 2011; Tzakou et al., 2007). The pollen contains free amino acids like phenylalanine, methionine, glutamic acid, prolin etc.

The Pods of Cassia fistula contains proanthocyanidin with flavin-3-ols (epiazelechin and epicatechins) units specifically the one having 2S- configuration together with common flavin-3-ols, proanthocyanidins like procyanidin B_2, catechins **(33)** etc. There is the highest level of phenolic compounds in pods. Triterpene and diterpene like norprimer-8(9)-ene-15-one, they also contain apolar compounds like 5-nonatetracontanone, 2-hextriacontanone, tricontane, 16-hextriacontanol, fistulic acid **(34)**, 3-formyl-1-hydroxy8-methoxy-anthraqiunone (Bahorun et al., 2004). It contains fistulic acid **(34)** anthraquinone which is a colouring matter (Agrawal et al., 2009).

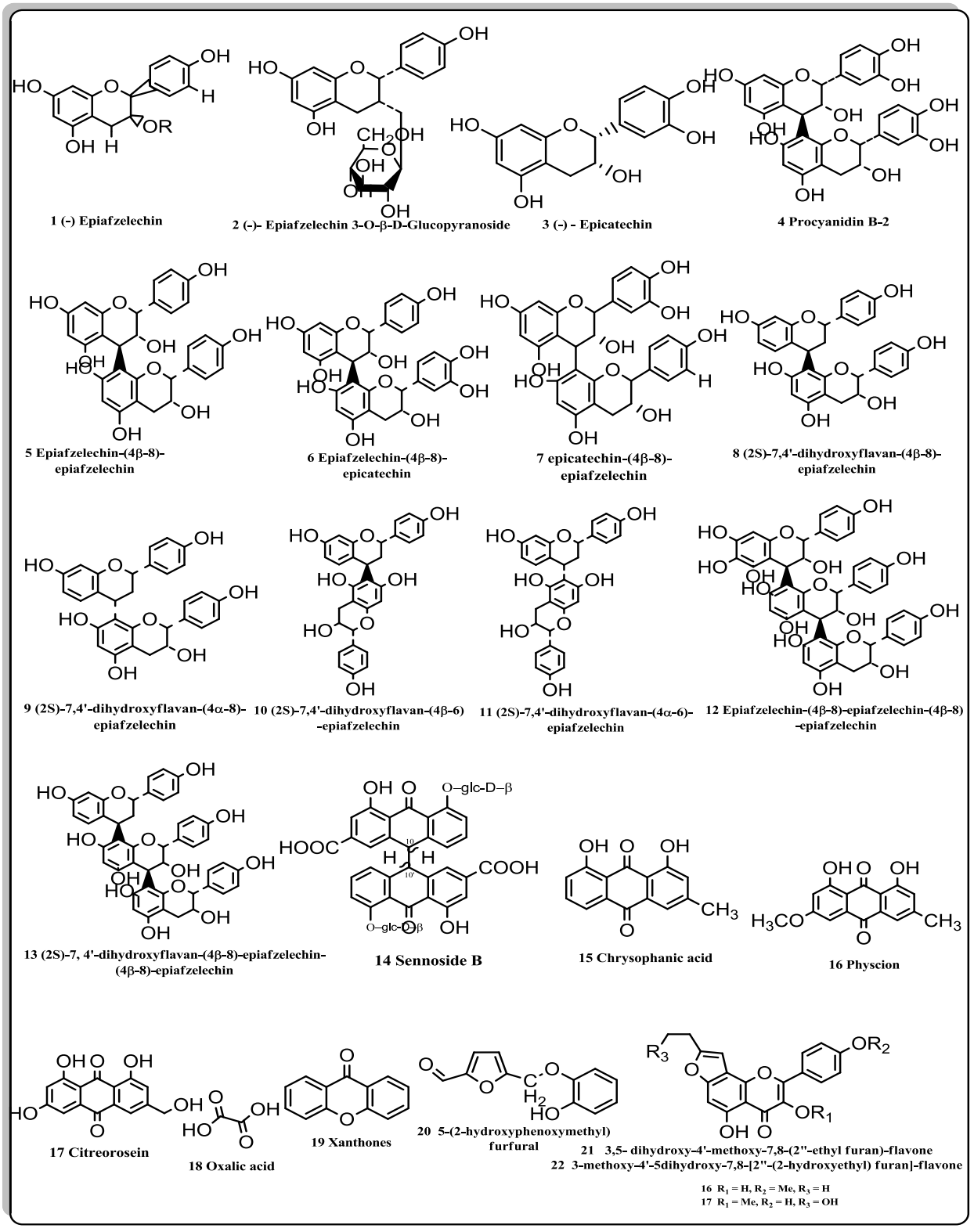


Figure 8 : Chemical constituents of *Cassia fistula* L.

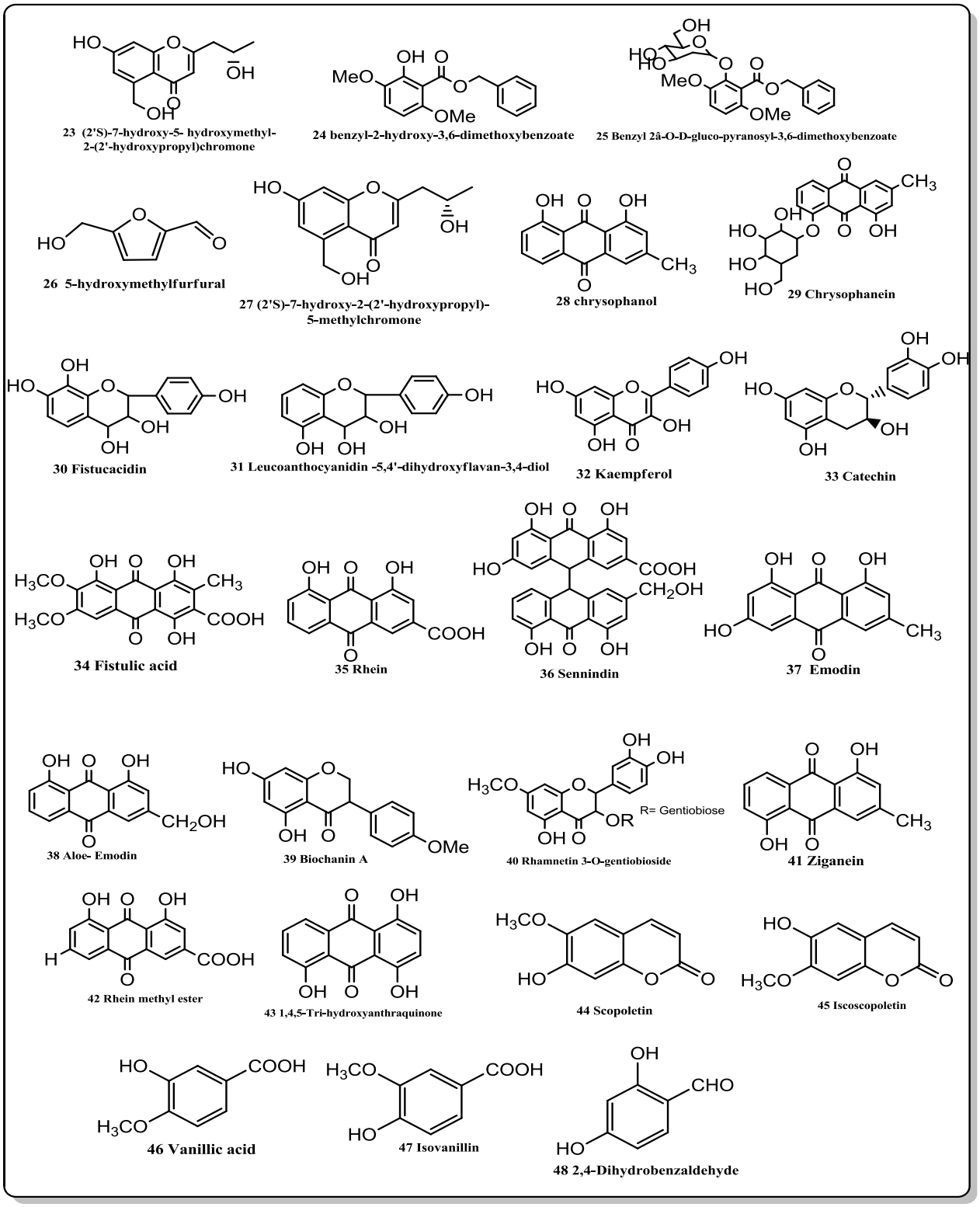


Figure 9: Structures of Chemical constituents of *Cassia fistula* L.

The pods contain anthraquinones in both aglycones and glycosides which are the active laxative form, while rhein **(35)** is a major compound. The contents of total anthraquinone glycosides in the ripe pods and in the leaves were found within a range of 0.21-0.67% (average 0.44%) and 0.05-0.74% (average 0.32%) dry weight, respectively. The contents of rhein (**35**) in the ripe pods and in the leaves of C. fistula determined by TLC-densitometric method were 0.05-0.14% (average 0.09%) and 0.002-0.29% (average 0.12%) dry weight, respectively (Gritsanapan & Nualkaew, 2008).The pods of Cassia fistula contain beta-sitosterol with an oil isoprenoid also (Bhalerao & Kelkar, 2012). It also contains sucrose, 31.3; fructose, 26.2; and glucose, 42.5% and high concentration of potassium (1809mg/100g). The Fistulic acid **(34)**, 3-formyl-1-hydroxy-8-methoxyanthraquinone, sennidin **(36)**, aloin, emodin **(37)**, aloe-emodin **(38)**, physcion and chrysophanic acid are also present (Dave & Ledwani, 2012).An isoflavone known as Biochanin A **(39)** was reported 2009 by Sartorelli et al. from the friuts of plant, which is found responsible for anti-parasitic activity.

Table 2: Polyphenolic contents and antioxidant activities (FRAP and TEAC values) in *In vivo* vegetative and reproductive organs of the total extracts of *C. fistula* and in vitro callus extract harvested after 35 days of culture (Bahorun et al., 2004).

Plant organ/Extract	**Total phenolics a**	**Total flavonoids b**	**Total proanthocyanidins c**	**TEAC d**	**FRAP e**
Young leaves		9	2	98	51
Old leaves	11	6	3	102	64
Twigs	12	3	2	93	64
Bark	9	4	2	157	95
Flower buds	13	8	20	893	380
Flowers	44	8	14	453	317
Pods	32	14	21	992	811
Callus (Day 35)	54	-	17.6	853	655
	31.4				

a-mg gallic acid equivalent/ g dry weight. b-mg quercetin equivalent/ g dry weight. c-mg cyanidin chloride equivalent/ g dry weight. d-mol/g dry weight. e-in units of µmol Fe(II)/g dry weight.

The Roots The chemical constituents in the roots of Cassia fistula L. were investigated with column chromatography. Nineteen compounds including seven anthraquinones, chrysophanol, physcion, emodin, aloe-emodin, 11-acetyl-aloe-emodin , rhein, citreorosein ,four flavan-3-ols, (-)-epiafzelechin, (+)-afzelechin , (-)-epicatechin, (+)-catechin; three sterols, a mixture of β-sitosterol and stigmasterol, β-sitosterol-3-O-β-glucopyanoside; one triterpene, lupeol; and four glycerides, a mixture of glycerol-1-tetraeicosanoate and glycerol-1-pentaeicosanoate, trimyristin and glyceryl trilinolate were determined by spectroscopic analysis (Kuo et al., 2003). Roots also contain rhamnetin 3-O-gentiobioside **(40)** (Vaishnav & Gupta, 1996). It also has 7-methylphyscion, betulinic acid and β-sitosterol, emodin, chrysophanic acid, barbaloin, fistucacidin (Danish et al., 2011; Dave & Ledwani, 2012). Recently two more compounds have been isolated from root bark of the plant. 4-dodecyl-1,3,8-trihydroxyanthraquinone and hexacosanoyl quinate along with some long chain fatty acids like heptacosyl eicosanoate, palmitic acid and one triterpene and lupeol (Jain, Jain, & Jain, 2013).

The Pulp of fruit contains 2.12% of total phenolic content (Siddhuraju et al., 2002). Rhein present on pulp is considered as major anthraquinone derivative in pulp, while other anthraquinones like citreorosein, ziganein **(41)**, rhein methyl ester **(42)**, 1,4,5-Tri-hydroxyanthraquinone **(43)** and other polyphenols, coumarins like scopoletin **(44)**, isoscopoletin **(45)**, vanillic acid **(46)**, isovanillin **(47)**, 2,4-dihydrobenzaldehyde **(48)** are also present in it (Bahorun et al., 2004; Leea et al., 2001).

Table 3: Content of rhein (one of the major constituent) in different morphological parts of *Cassia fistula* L. (Shailajan *et al.*, 2013).

Parts of *Cassia fistula*	**Content in mg/g (mean±SD, n=7)**
Leaves	2.21±0.18
Fruit pulp	1.90±0 .17
Flowers	0.89±0 .12
Seeds	0.19±0.02

Extracts of fruits contains amino acids like aspartic acids, glutamic acids and lysine of total amino acids. These have high concentration of sugar as sucrose, fructose, glucose and few other macro mineral nutrients. There are micronutrients also present in it like potassium, calcium, iron and manganese. The ripe fruits of C. fistula were processed to obtain sennoside B, used as a laxative in modern medicine. Extraction by dilute alcohol yielded an active concentrate containing a higher percentage of sennoside B (4.23%) with lower ash value in comparison with a concentrate extracted by water alone (0.74%) (Chowdhury et al., 1996). It also has sugar, gum, astringent matter, gluten, colouring matter and water (Danish et al., 2011). Fruit pulp contained proteins (19.94%) and carbohydrates (26.30%); arginine, leucine, methionine, phenylalanine, tryptophan, aspartic and glutamic acids (Danish et al., 2011).

CHAPTER – 3

MATERIALS AND METHODS

Chapter – 3

Materials and Methods:

3.1 Plant material

Plant material was identified with the help of Dr. Attar Singh (Taxonomist) Dept. Central University of Punjab, Bathinda. Collection of leaves was done in the month of August 2013; from the University campus, bathinda region latitude 30^{o}14' N, Longitude 74^{o}57'E and Altitude 220 - 205 meters above sea level. Apart from Shade drying, tray dryer was used for drying of leaves. Extraction was performed using Aspirator.

Figure 10 : Drying the leaves of *Cassia fistula* L. in shade and via using Tray dryer.

Figure 11 : Aspirator used for Extraction of Powdered leaves of *Cassia fistula* L.

The chemicals and solvents were purchased from Sigma-Aldrich, Loba-Chemie Pvt. Ltd., S.D. Fine Chemicals, Sisco Research Laboratory, and consumed without refining. Solvents used were Petroleum ether, Ethyl acetate, Methanol and Water. Water used for the experiment was free from ions and is double distilled by RPMI1640.

3.1.1 Instruments

Instruments Used	**Company**	**Purpose**
Rotavapor	Ilmvac	Drying extracts/fractions
Hemocytometer	Invitrogen	For counting of cells
Incubator	Galaxy, New Brunswick	Incubation
Centrifuge 5430 R	Eppendorf, Germany	Centrifugation
Laminar air flow	Macro Scientific Works	For aseptic condition
UV-VIS Spectrophotometer	Shimadzu	Absorption studies
Inverted microscope	Magnus, Olympus	Visualization of the cancer cells

Glasswares like Round bottom flasks, Aspirator, beakers, Erlenmeyer flasks were used of Borosil. Filtration assembly, Vacuum pump, Buchner flask, Sintered glass – Buchner funnel was used for performing dry column to prepare different extracts. Whatmann filter paper was also used. DMEM, Penicillin/ Streptomycin antibiotic solution, phosphate buffer saline and fetal bovine serum media which were used to culture cancer cell lines were purchased from HiMedia. MTT dye used for MTT assay was also made available from HiMedia. DMSO, extrapure AR was purchased from SRL.

3.2 Cancer cell lines

Various cancer cell lines (lung/breast) were used to establish the anti-proliferative potential of the extracts using in vitro MTT assay. All the cell lines were procured from national cell repository located at NCCS, Pune.

3.2.1 MCF-7 (breast cancer cell lines)

This cancer cell line was substantiated in 1973 at Michigan Cancer Foundation (on the basis of which cell lines name is abbreviated) by Soule and colleagues. These are adenocarcinoma cell lines, which were derived from an old women's pleural effusion; who was suffering from metastatic disease. These are in general, are considered as the the model cell lines for ER (estrogen receptor) positive breast cancer. MCF – 7 is considered as the definitive model for the study of tumor response to endocrine therapy, estrogen receptors, progesterone receptors, glucocorticoids and androgens (Levenson & Jordan, 1997).

3.2.2 A549 (lung adenocarcinoma cell lines)

A549 cells were discovered in 1972. It is reference for adenocarcinoma cell lines. These have the adherent property. These were derived from a 58 years old Caucasian male from his cancerous tissue of lung. They are morphologically determined as alveolar basal squamous epithelial cells. Their function is to manage the water and electrolytes diffusion across alveoli of lungs. These are adherent cells and form a monolayer, in vivo. These are non-metastatic cells (Forest et al., 2005). These cells synthesize lecithins along with high concentration of de-saturated fatty acids, and are important for the maintenance of phospholipids in cells and are also utilized in cytidine-diphosho-choline pathway. In in-vitro studies these are used as pulmonary epithelial cells model for drug metabolism studies. These are used in the study of viral infections, asthma, damages linked to asbestos, smoking related emphysema also.

3.2.3 H-460 cell lines (Large cell lung carcinoma)

These are large cell lung carcinoma cell lines. These are also derived from the pleural effusion fluid of lungs from a patient suffering with large cell lung cancer in 1982. These are epithelial cells with adherent property. These cells have metastatic property (Forest et al., 2005).

3.3 Extraction of Plant Materials

3.3.1 Collection and preprocessing of plant

Leaves of Cassia fistula were collected from the University campus, at Central University of Punjab, Bathinda in the month of August, 2013. Collected leaves were cleaned and then dried in the shade and also in tray dryer and later ground to powder form. The total weight of the powder was 4.3 kg.

3.3.2 Preparation of extracts

The 2 kg of powder leaves was packed in aspirator and subsequently subjected to extractions using different solvents with increasing order of polarity. The percolation was carried out using different solvent system as petroleum ether (2x2.2 L), ethyl acetate (2x1.8 L), methanol (2x 1.5 L), methanol: water 7:3, v/v (2x1 L) and the menstrum collected was concentrated and dried to powder form using rotavapour under reduced pressure to render petroleum ether (34 g), ethyl acetate extract (71 g), methanol extract (84 g) and aqueous-methanolic extract (150 g).

3.4 Procedures and assay performed for *In-vitro* studies

3.4.1 Preparation of Stock Solution for *in vitro* study

The extracts were subjected to in vitro for their cytotoxic activity showed results in varied range. The stock solution of each extract used for in vitro study was prepared by dissolving extracts in DMSO to form final concentration of 1mg/ml. Two concentrations which include 10μg/ml and 50μg/ml were used to treat the cells by suitably diluting stock solution.

3.4.2 Culturing of the Cell Lines

All the cancer cell lines were treated in the appropriate medium (DMEM). Trypsinization was done by adding trypsin for the purpose of detachment of cancer cell lines. Subsequently the trypsin was inactivated by adding 1mL media containing serum. Centrifugation was done using 1200 rpm of speed at 37˚C for 10 minutes for harvesting the cells. Supernatant was disposed and re-suspension of the cell pellet

was done using 2 mL of the media. The cell number was counted using automated cell counter instrument. The cells were transferred to fresh media every three days. The cancer cell lines were already cultured. The maintenance of cultured cell lines was done in 25 cm^2 or 75 cm^2 flasks containing DMEM medium supplemented with 10% fetal Bovine serum (FBS), 1X antibiotic solution (that is; 50 U/ml penicillin G, 50 µg/ml streptomycin sulphate) and afterward incubated at 37 ˚C in a humidified atmosphere containing 5% CO_2 and 95% humidity. Subsequently the cells were sub cultured in 25 cm^2 flasks which become necessary when cell lines have attained 70-80% growth. The reagents necessary for this procedure were placed in water bath which is maintained at the temperature of 37 ˚C for 10-15 minutes before sub-culturing was done. Trypsin was added during the sub-culturing and after the interval of 5 minutes 1 mL of serum containing media was incorporated to cease the action of trypsin. Afterwards cells were transferred to centrifuge tubes of 15 mL capacity and centrifugation was done for 10 minutes at the speed of 1200 rpm. The supernatant was disposed and the pellet was again re-suspended in complete media. The cell lines were transferred to fresh media every three days. Approximately, 8000 cells per well of 96 well plate were seeded for cytotoxicity analysis followed by extracts treatments as indicated in results section. For treatment purposes, extracts were suspended in DMSO which was further diluted in complete cell culture media. After the incubation period of 24 hour the cells were treated with extracts in triplicate with concentration of 10µg/ml and 50µg/ml and cells were incubated for 48 hours (Baviskar et al., 2013). Cells prepared were then washed with 1x PBS and mixed with 100µl per well of MTT (5mg in 10ml of 1x PBS) and incubated at room temperature in dark for 4 h to let production of formazon crystals. Each well then mixed with 100µl of DMSO to dissolve the crystals followed by analysis using microplate reader at 570nm. Results were then plotted in graphs to calculate cytotoxic potential and IC_{50} values (Baviskar et al., 2013; Zhang & Webster, 2013).

Three human cancer cell lines were incorporated that are having different origin, tumourogenicity and morphology for study (Forest et al., 2005; Levenson & Jordan, 1997). H – 460; the large cell lung carcinoma cell lines with adherent and metastatic properties (Forest et al., 2005). To study the outcome values of cytotoxicity assay, their readings were plotted in bar graph form with “percentage survival” being taken on

ordinate axis versus Vehicle control and extracts with different concentrations on abscissa (Figure 9). The microplate readings were converted into percentage survival and plotted with vehicle control as 100% viable cells. And the obtained readings were scrutinized for the activity.

3.5 *In silico* studies

Computational analyses were carried out on windows 7 professional platform running on HP-Work Station K800 series with Intel Xeon processor and 8 GB of RAM. The molecular docking simulation study was utilized to determine possible binding modes of a ligand to the active site of a receptor.

3.5.1 Ligands preparations

Library of molecules reported in Cassia fistula was created using literature and there structures were downloaded from pubchem while some of the structures which were unavailable in library were drawn using Maestro 9.6 (Release, 2013) from their respective referenced articles (Kashiwada et al., 1990; Kuo et al., 2002; Yadava & Verma, 2003; Satoshi et al., 1988) to investigate their anticancer potential. Ligands were converted to 3D structure from 2D using “LigPrep” version 2.5 (Sastry et al., 2013) (Maestro, 2012). “LigPrep” produces a single, low energy, 3D structure with correct chiralities for each input structure, hydrogen added and orientation of various functional groups was performed. During the performance of this step, chiralities were determined from 3D structure and original states of ionization were retained. “Ligprep” application of the Maestro 9.6 utilizes OPLS-2005 force field (Sastry et al., 2013). Gefitinib (CID 123631) is used here as a standard inhibitor for EFGR kinase mutations; as it is used in first line treatment for NSCLC and is an important target in breast cancer (Gutteridge et al., 2005; Gridelli et al., 2011). Wortmannin is a standard inhibitor of mutations in PI3 kinase also it is being tested for its dual inhibition potency against mTOR (Pavlidou & Vlahos, 2014; Yuan & Cantley, 2008). For Akt kinase; AZD5363 (CID 25227436) and for mTOR; Sirolimus (CID 23724530) were used as the standard inhibtors.

3.5.2 Protein preparation

The three dimensional crystal structures of EGFR (2ITY) (Yun et al., 2007), PI3K (3S2A) (Nishimura et al., 2011), Akt (3MV5) (Freeman-Cook et al., 2010) and mTOR (4DRI) (Hausch et al., 2013) were retrieved from database of protein data bank RCSB (Research Collaboratory for Structural Bioinformatics). Protein structure with polar hydrogen was prepared using the protein preparation wizard in Maestro 9.6 (Maestro, 2012). In this step, bond orders were assigned, all hydrogen were added, bonds to metals were deleted, formal charges were set on the metal, neighboring atoms and water molecules were deleted that were more than the 0 Å specific distance and in case of 4DRI water molecules were deleted from the 5 Å specific distance. Any missing disulphide bonds were added. The H-bonds were optimized using “protassign” at pH 7. With generated Hetero states options, prediction of ionization, and tautomeric states of the het group at pH 7 was achieved. In protein preparation, reorienting hydroxyl group, water molecules, and amino acids lead to the optimization of hydrogen bond network. Refinement of the structure was the final step in the protein preparation, with the help of restrained minimization. It was initiated in the imperfect minimization with the 0.3 Å RMSD for the minimization OPLS-2005 force field (Jacobson et al., 2004; Singh & Bast, 2014). All bound ligands (small molecules and BH3 peptides), waters beyond 5 Å and ions, molecules and heteroatoms were removed from the complexes, bond order assigned, disulfide bonds created and zero order bonds to metal charge fixation are incorporated to raw PDB structure (Sastry et al., 2013). The 3MV5 is a monomer with resolution of 2.47 Å. 4DRI is a heteromer with two chains A & B with active site being present in A chain. It has resolution of 1.45Å. The domain used here for the protein is a fragment of the rapamycin binding domain.

3.5.3 Receptor Grid Generation

Grid generation at a particular site in the protein after analyzing the receptor; is the key step to perform Molecular Docking and MMGBSA studies. It helps to specify the receptor pocket; where docking is required to be performed. By adjusting the different parameters, an environment virtually similar to in vivo conditions; favorable interactions between ligands and receptor can be observed. Grid is generated at place of pre-

existing ligand using default settings for 3mv5, 3s2a and 2ity. The grid was generated in which Van der Waals scalling 1.0, with partial atomic charge cut-off of 0.25. The X, Y, Z-ranges for receptor setup was 28, 28, and 28 with n-sites value being 125 and B size was 1.0.

3.5.4 Molecular Docking Studies

The protein-ligand docking studies were performed using maestro suite of version 9.6. Glide ligand docking jobs require a set of previously calculated receptor grids and one or more ligand structures. Grid was generated and then molecules were docked using glide docking module. Ligands were prepared using ligprep wizard of maestro suite. In this step hydrogen addition, charge fixation & orientation of various functional groups, conversion of 2D structure to 3D structure, bond length and angle correction, appropriate chirality assignment and other alterations were incorporated into the ligand molecule at last, OPLS_2005 force field was applied for minimization and optimization purpose. Single conformation was generated for each ligand for docking. Glide ligand docking was performed using XP docking in which ligand was taken as flexible. Sample nitrogen inversions and sample ring conformations were taken into account. Bias sampling of torsions was one only for the amides and non-polar conformations were penalized. Epik penalties were added to the docking score.

3.5.5 Protein-Ligand Binding Studies

The Prime MM-GBSA is used for binding energy estimation by uploading Maestro pose viewer file. MMGBSA dG bind as a major descriptor was taken into consideration which depicts the binding energy of the receptor and ligand as calculated by, Prime energy, a molecular mechanics and implicit solvent energy functions (kcals/mol) = Prime energy (optimized complex) - prime energy (optimized free ligand) – prime energy (optimized free receptor) (Greenidge et al., 2012). Ligand strain energy that is a prediction of the energetic penalty due to strain between the ligand in the complex and the ligand in the free state based on the difference in Prime Energy (kcals/mol). = PrimeEnergy (Ligand geometry from optimized complex) – PrimeEnergy (Optimized free ligand). Rec (receptor) Strain Energy that is a prediction of the energetic penalty

due to strain between the receptor in the complex and the receptor in the free state based on the difference in Prime Energy (kcals/mol). = PrimeEnergy(Receptor Geometry From Optimized Complex) - PrimeEnergy(Optimized Free Receptor) . The prepared ligands were docked on the respective protein to study their binding interactions. Binding energy was calculated for them using MM-GBSA that is a thermodynamic statistics for Bimolecular Systems. MM-GBSA can be stated as Molecular mechanics – the generalized born model and solvent accessibility method (Greenidge et al., 2012; Jacobson et al., 2004; Mobley & Dill, 2009).

3.5.6 ADMET properties studies

Prepared ligands were neutralized and processed in QikProp module to check the ADMET properties. Determination of these properties provides significant information about the moieties pharmacokinetics and pharmacodynamics characteristics (Singh & Bast, 2014; Manikandan et al., 2011). These are accountable for 60% rejection of all drugs in clinical phases (Patel et al., 2013). QikProp predicted considerable properties like Molecular weight (MW), permeability through MDCK cells, log IC_{50} value for blockage of K^+ channels (QPlogHERG), gut-blood barrier (QPPCaco) and violations of the lipinski's rule of five. Knowing these properties helps in elimination or modification of molecules with poor ADME properties and pilots to major savings in research and development outlay (Hamsa et al., 2013; Sudha et al., 2011).

CHAPTER – 4

RESULTS AND DISCUSSION

Chapter-4

Results and Discussion

Before performing molecular modeling studies on reported molecules of Cassia fistula the anti-proliferative activity was tested to make our study more selective and specific to evaluate the anti-cancer potential of extracts. Therefore, biological screening through cytotoxicity assay (MTT) on different extracts of Cassia fistula was performed. Extracts were prepared with the choice of solvents in the increasing order of their polarity. Assay was performed on three different cancer cell lines MCF-7, H460 and A549 namely.

4.1 Extracts obtained

The different extracts have been prepared using Petroleum Ether, Ethyl Acetate, Methanol, and Hydro-Methanol as solvents. Plant material was extracted two times with each solvent. The detailed description of dried extracts obtained is as mentioned in Table 4.

Table 4: The amount and percentage of extracts obtained in the study.

S.no.	Extracts Type	Amount obtained (in grams)	Percentage of extracts obtained*
1.	Petroleum Ether	34 g	1.7%
2.	Ethyl Acetate	71 g	3.55%
3.	Methanol	84 g	4.2%
4.	Hydro-Methanol	150 g	7.5%

* have been calculated by formula wt. of extract/ formula wt. of total plant material used *100.

4.2 *In-vitro* cytotoxicity studies

The antiproliferative effects of Cassia fistula were observed using MTT assay. The multiple concentrations of all the four extracts SVA-1 (Petroleum Ether extract; used only for defatting readings not shown), SVA-2 (Ethyl acetate), SVA-3 (Methanol) and SVA-4 (Hydro-methanol). Effective doses were calculated using dose response curve.

4.2.1 Antiproliferative activity of extracts against MCF-7, A549 and H460

In MCF-7 cell line, the tested extracts showed potent cytotoxic activity near 50µg/ml in all samples, especially in SVA-2 and SVA-4. At 10µg/ml, SVA-3 showed excellent

cytotoxic activity which was comparable to the inhibitory activity at the concentration of 50µg/ml in case of SVA-4. In A-549 cell lines, SVA-4 showed excellent inhibitory activity at 10µg/ml as well as 50µg/ml, which was better than SVA-2 and SVA-3, which display 50% inhibitory potential. For H-460 cell line, SVA-4 at 50µg/ml concentration showed noteworthy response.

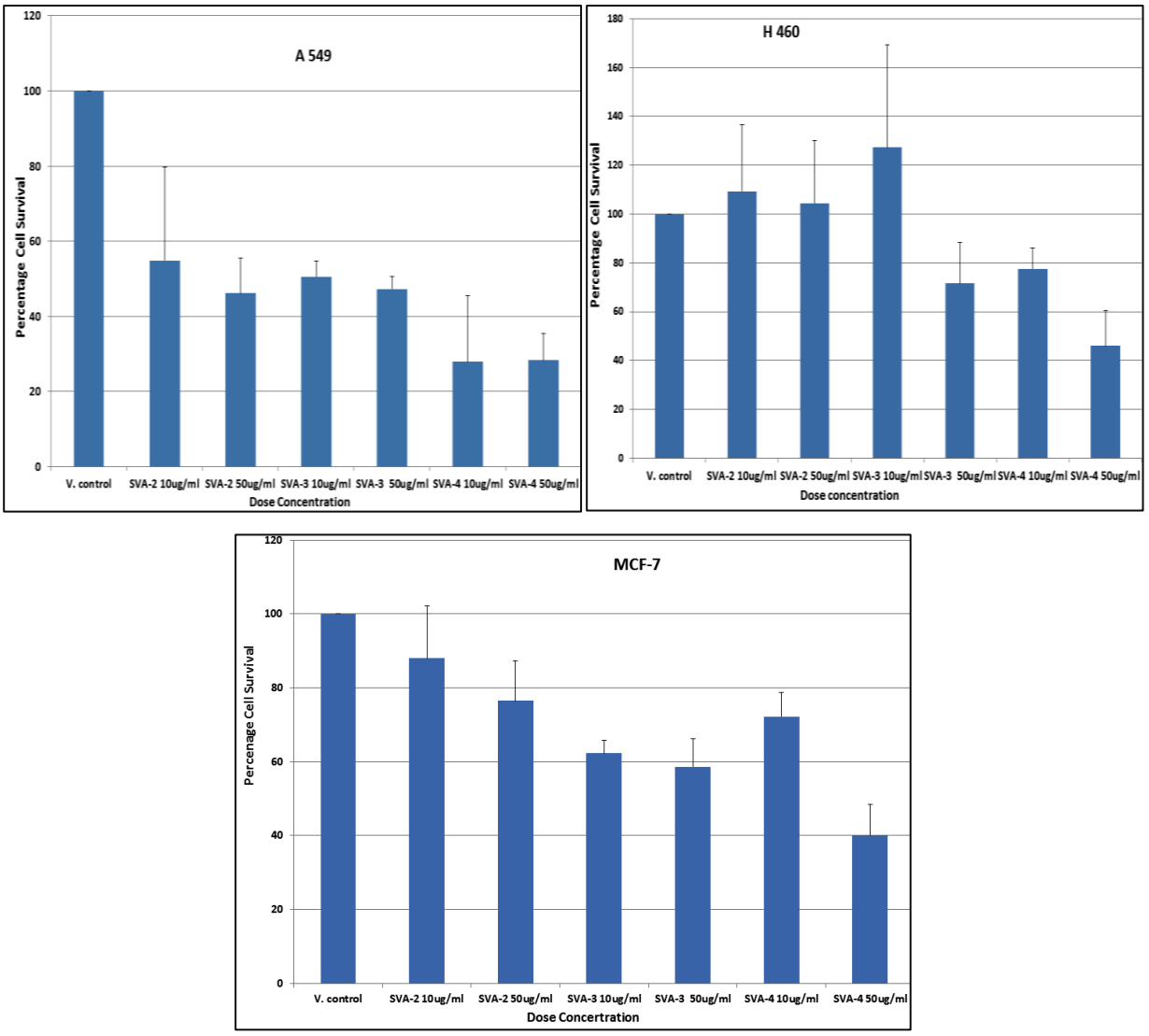


Figure 12: Bar graphs of cytotoxicity studies performed on cell lines A549 (lung adenocarcinoma), H460 (large cell lung carcinoma) and MCF-7 (breast cancer cell line).
It depicts percentage survival (% viability) of cell at different concentrations of the extracts in comparison to standard that is vehicle control. Vehicle control here used is DMSO. At concentration of 50µg/ml all the extracts are showing least cell viability that is maximum cytotoxicity for cancer cells.

4.2.2. IC_{50} values of extracts:

As stated earlier, the SVA-4 has provided the finest cytotoxicity results in the assay among all the cell lines. The most appropriate IC_{50} value depicted by SVA-4 was <10µg/ml, reported in case of A549 cell line. Apart from SVA-4, SVA-3 and SVA-2, has also indicated note- worthy IC_{50} value of 30µg/ml and 42µg/ml, respectively. In MCF-7 and H460, low cell viability with 38µg/ml and 42µg/ml of IC_{50} value, was observed with this extract.

Table 5: Extracts showing IC50 values for different cell lines

S. no.	Cell line	Concentrations	IC50 value
1.	MCF-7	SVA – 4	38 µg/ml
2. (a)	A 549	SVA – 2	42 µg/ml
2. (b)		SVA – 3	30 µg/ml
2. (c)		SVA – 4	<10 µg/ml
3.	H 460	SVA – 4	42 µg/ml

SVA2- ethyl acetate extract, SVA3-alcoholic extract, SVA4-Hydroalcoholic extract.

A549-adenocarcinoma lung cancer cell line, H460-large cell lung cancer cell line, MCF7-breast cancer cell line.

IC50 value of prepared extracts of leaves of Cassia fistula is given in units of µl/ml.

Vehicle control used in assay was DMSO.

As it can be observed from the results mentioned above, hydro-methanolic extracts (SVA-4), methanolic extract (SVA-3) and ethyl acetate extract (SVA-2) are showing estimable cytotoxic activity particularly at the dose of 50µg/ml against all the cell lines. It can be thus concluded, that such results could be due to the combined activity of the various natural moieties present in these extracts or these results could be the outcome of the collective effect of few moieties from the plant extracts with anticancer properties. As per the National Cancer Institute (NCI), the criteria of cytotoxicity of crude extracts is established as the IC_{50} value of <30 µg/ml after exposure of 72 hours and extracts with cytotoxic values <20µg/ml are considered to carry potential cytotoxicity (Geran et al. 1972). Appertaining to the above statement, the cytotoxicity observations and recordings made in the present study were after 48 hrs, where the A-549 cell line showed noteworthy results within the provided range by NCI, particularly for methanolic and hydro-methanolic extract that are 30µg/ml & <10µg/ml. In MCF-7 cell line, observations were recorded near the mentioned range in hydro-methanolic

extract i.e. 38μg/ml (Table 5) after 48 hrs, and 42 μg/ml with hydro-methanolic extract (Table 5) for H-460 cell line.

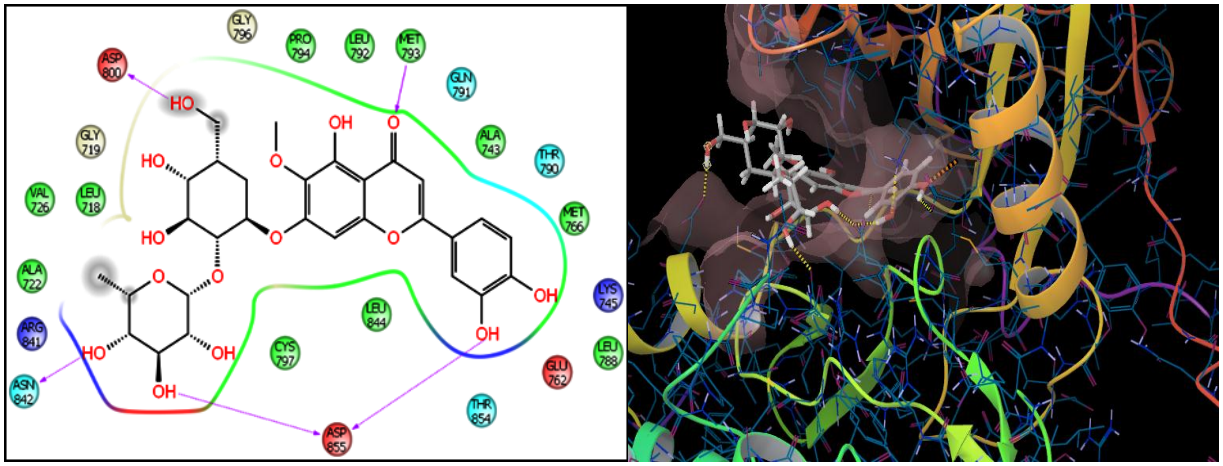
Thus, the results are portraying a clear picture for the SVA-4, that it possesses the most potent anti-proliferative property for all the three cell lines out of all the extracts. Particularly near the concentration of 50μg/ml all the extracts are possessing noteworthy cytotoxic effects. SVA-4 is signifying the best IC_{50} value that is <10μg/ml (Table 5) for the adenocarcinoma lung cancer cell line A549.

4.3 *In silico* studies

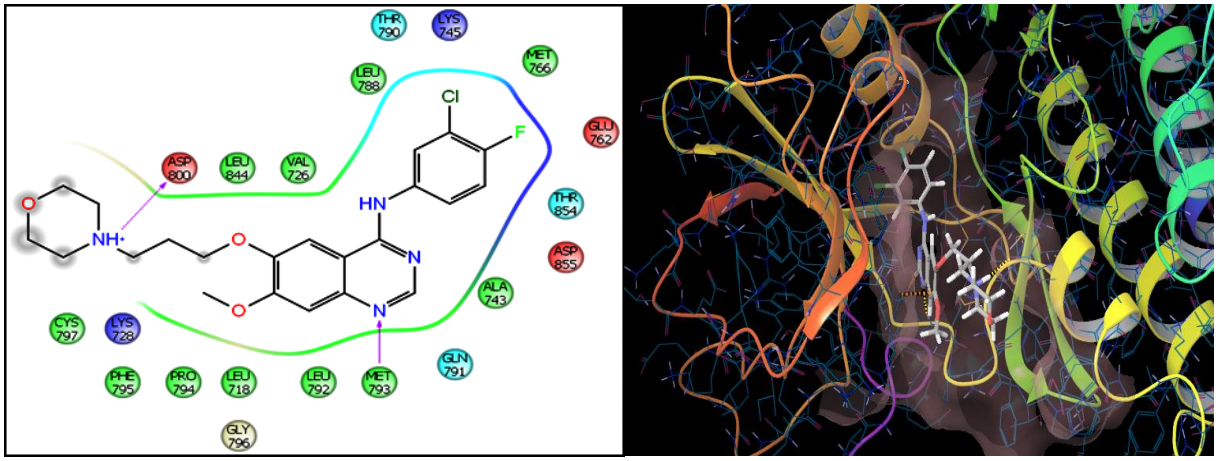
4.3.1. Protein – Ligand binding interactions of EGFR (2ITY)

EGFR kinase is a transmembrane tyrosine kinase receptor belonging to HER family of receptor. EGFR overexpression is generally observed in LC and BC diagnosis. Hence, it is aimed in their treatments. As per the result obtained after performing docking and MM-GBSA on EGFR (2ITY), 5,3,4-tri-hydroxy-6-methoxy-7-O-α-L-rhamnopyranosyl-(1→2)-O-β-D-galactopyranoside (ligand **26**) (Figure 13a) came out as the moiety with best binding energy (dG bind score - 98.787 kcal/mol) (Table 6) as compared to standard Gefitinib (Figure 13 b) having binding energy score of -86.559kcal/mol (Table 6) . From the data mentioned in Table 6, it was observed that ligand **26** is having far better interaction with the receptor at the kinase domain as compared to gefitinib. Ligand **26** has shown sturdy H-bonding with a score, -3.9782 kcal/mol (Table 6) while, standard inhibitor of EGFR kinase that is gefitinb has far weaker H-bonding interaction of -1.4467 kcal/mol. Further, H-binding is observed in ligand **26** receptor grid site on its side chain through Asp 800, Asp 855 (involved in H-bonding from two side chains of molecule) and Asn 842 as illustrated in Figure 13 along with an H-bonding through the hydroxyl group present in pharmacophore part of molecule through Met 793. Gefitinib on the other hand, is binding through Asp 800 and Met 793 only. Because of this strong H-bonding interaction the ligand 26 has shown good binding affinity for the receptor. Ligand **26** has indicated attractive coulomb forces interaction with value of -49.05kcal/mol. This means as the bond formation took place between the molecule and the kinase domain of receptor, there were atomic interactions with attractive forces

to an appreciative extent. While gefitinb, has provided comparatively weaker columbic interactions with a value of -32.23kcal/mol.



(a)



(b)

Figure 13: The binding energy interactions between (a) 5,3,4-tri-hydroxy-6-methoxy-7-O-α-L-rhamnopyranosyl-(1→2)-O-β-D-galactopyranoside (Ligand 26) and EGFR kinase domain and (b) Gefitinib and EGFR kinase domain

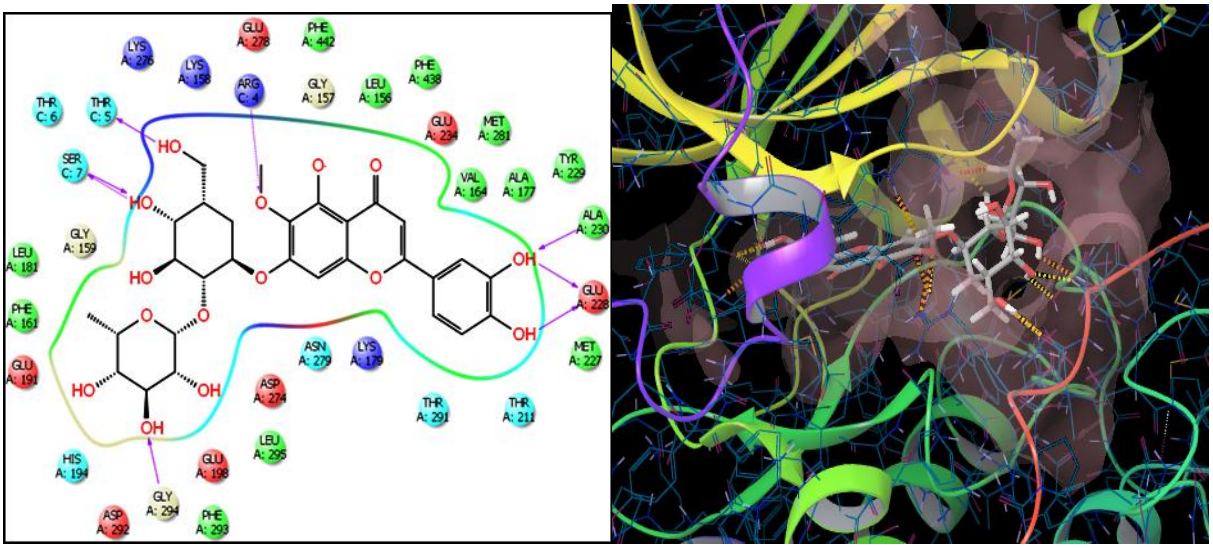
Even hydrophobic bonding has been depicted as comparatively better for ligand **26** than gefitinib. The EGFR is a transmembrane protein with different kinase domains classified into subtypes ERBB/HER, ERBB1/HER1, ERBB2/HER2 and few more. The major mutated domain is ERBB1 which is picked for current study along with its standard inhibitor gefitinib. The ligand **26** has shown the observable binding energy score of -98.78 kcal/mol as compared to standard Gefitinib (-86.55kcal/mol). As discussed in the result section, there is quite observable variations in the other descriptive values like H-bonding, van der Waals forces, columbic forces. The gefitinib

is a 4-anilinoquinazoline; its quinazoline ring binds in ATP binding pocket that is present near to the main chain amino acid residue Met 793 and form a hydrogen back bonding with it. The Met 793 lies in the hinge region of the kinase that connects the C lobe and N lobes of the protein. Gefitinib forms single H-bond, similar interaction is being observed by Ligand 26 also but yet stronger due difference of atom that is involved in binding. In gefitinib the H-bonding is done by “N” of morpholino ring of geftinib while in ligand **26** it is done by “O” of the rhamnopyranoside ring of ligand **26**. Although the 3-chloro, 4-fluoro substituent is extending in hydrophobic region at the back cleft of the ATP- binding pocket and interacting with Lys 745, Leu 788and Thr 790, but Ligand 26 is extending in same hydrophobic pocket with a di-hydroxy aryl ring and at the place of 4-fluoro interactions, the hydroxyl group of aryl ring is interacting with Thr 854 and Glu 762. Interactions from this pocket; enhances further a step as compared to geftinib as the same hydroxyl group back bonding interactions advances to residue Asp 855. An extension of ring made in gefitinib structure was attachment of 6-propylmorpholino group which weakly interacts with solvent and Asp 800 residue by H-back bonding due to weak electron density. Here, on the contrary a striking difference has been observed in the binding interactions of ligand **26** the rhamnopyranoside and galactopyranoside ring if the ligand **26** extends dually in this region and along with forming H-back bonding Asp 800, it additionally interacting with Gly 719 present in the neighboring region. The rhamnopyranoside ring is interacting forming H-back bonding with Asp 855. Thus appears to conjoin the interactions between hydrophobic pocket and Cys 797, Asn842, Arg 841 and Leu 718. This is due to extension of structure of ligand **26** on the rhamnopyranosyl and galactopyranoside ring residue which is able to enter and orient more appropriately in the receptor grid hence improving the interactions with the solvent and electropositive and electronegative amino acid residues present in the pocket, whereas gefitinib in this region has very feeble interactions due poor structure extension. Although this extension of galactopyranosyl rhamnopyranoside ring may also improve the pharmacokinetic profile of the molecule as in gefitinib the propylmorpholino ring is used for the same purpose.

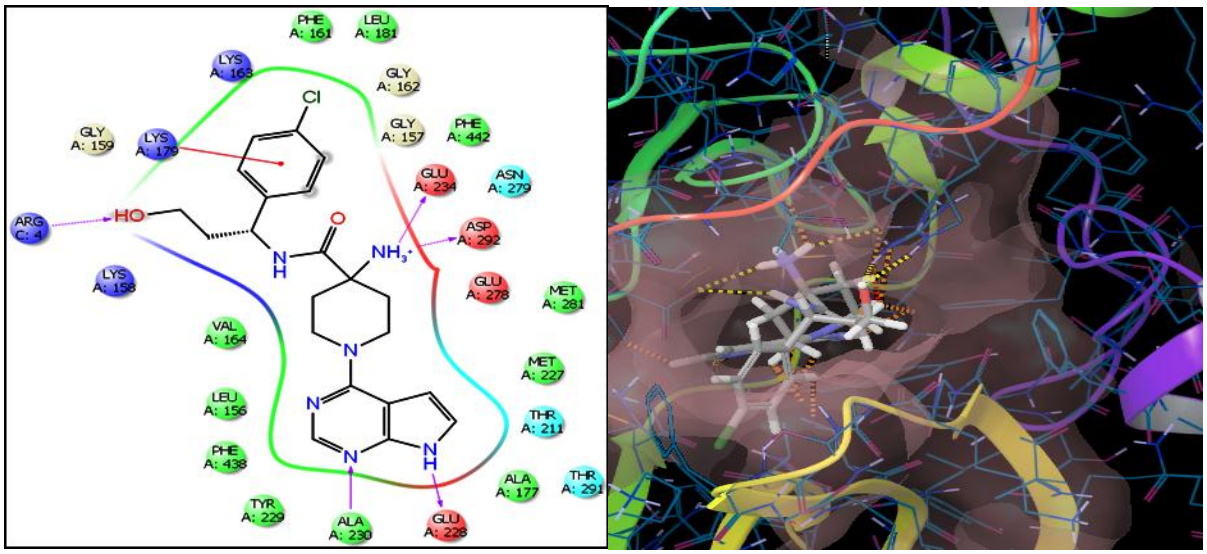
4.3.2 Protein – Ligand binding interactions of Akt kinase (3MV5)

Binding energy score of ligand **26** (Figure 14 a) was found to be -87.352kcal/mol (Table 6), while that of standard that is Azd5363 (CID_25227436) (-76.596kcal/mol) (Table 6) (Figure 14 b). The ligand **26** again has offered binding interactions with receptor that are better than the standard inhibitor drug for Akt kinase receptor. Ligand **26** presented H-bonding of value -3.9280 kcal/mol whereas AZD5363 showed it at -3.3035 kcal/mol. Ligand **26** offered H-bonding interactions with backbone at Gly A:294, Glu A:228, Ala A:230, Thr C:5, Ser C:7, while it was indicates H-bonding through side chains at Ser C:7, Arg C:4. It can be depicted from the readings of lipophillic binding energy, that ligand **26** is having noteworthy lipophillic interactions with protein. The lipophillic binding energy value has been observed as -50.6018kcal/mol (Table 6) while the standard Akt inhibitor showed -34.8066 kcal/mol. This portrays about the hydrophobic interactions that ligand **26** is having, with the kinase domain of receptor.

It can be observed from Figure 14 a. that there is presence of various hydrophobic amino acids around the moiety of ligand **26**, like [Leu A: (156,181,295)], [Phe A: (161, 442, 438, 293)], [Val A: 164], [Ala A: (177, 230)] and [Met A: (227, 281)]. Further the higher value of the binding energy score can also be attributed to the van der Waals forces of attraction present between amino acids present at the receptor grid and the ligand **26** moiety. The values of van der Waals forces binding were reported to be -64.105 kcal/mol while that of standard inhibitor was observed to be -39.470 kcal/mol This is again due to presence of hydrophobic amino acids around the moiety, which might have helped in the dispersion of charges and generation of dipoles that further enhances the van der Waals forces of attraction. Thus, the appropriate combination of positively charged; negatively charged and hydrophobic interactions along with H-bonding interactions made the ligand **26** to bind to receptor grid more efficiently than the standard inhibitor drug of Akt receptor. The serine–threonine kinase family member Akt kinase protein has crucial role in cell proliferation, survival, growth and other cellular processes. Mutations in this pathway are favourable for flourishing cancer. In the current study Azd 5363 is taken as the standard inhibitor for Akt kinase. Ligand **26** (dG bind score -87.35 kcal/mol) (Table 6) appears to show better binding interactions than Azd 5363 (dG bind score -76.59 kcal/mol) (Table 6).



(a)

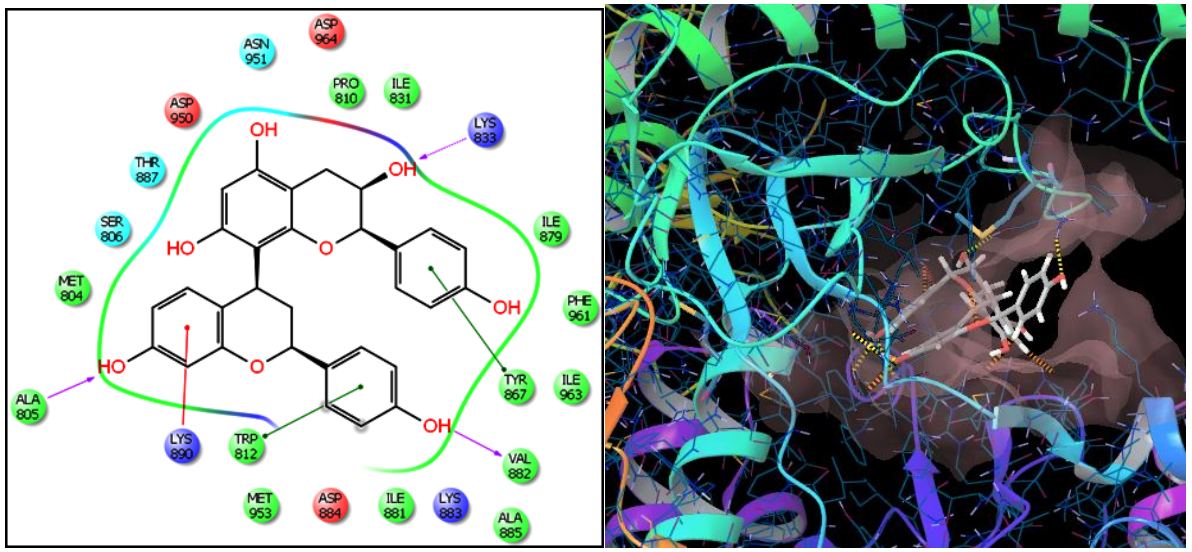


(b)

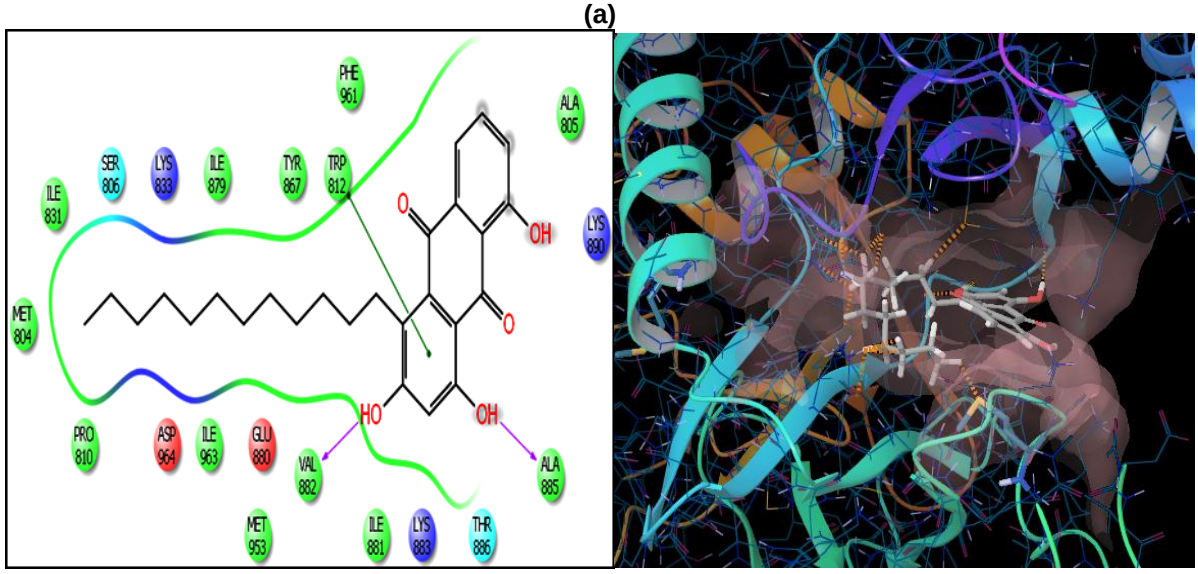
Figure 14: The binding energy interactions between (a) 5,3,4-tri-hydroxy-6-methoxy-7-O-α-L-rhamnopyranosyl-(1→2)-O-β-D-galactopyranoside (ligand 26) and Akt kinase domain and (b) AZD 5363 and Akt kinase domain.

Ligand 26 is showing similar interaction as Azd 5363 in the hinge hydrophobic pocket with Glu 228 and Ala 230, while ligand **26** is lacking the direct interaction with Glu 234 and Asp 292 due to which its H-bond score is lesser than Azd 5363. But due to extending form of structure enhanced hydrophilic and electrostatic interactions with electronegative (Asp 292, 274, Glu 234, 191, 198, 228), electropositive (His 194, Lys 158, 276, 159 Arg 4) and hydrophilic (Ser 7, Thr 5, Asn 279, Thr 291, 211) amino acid residues, it is showing better van der Waals and lipophilic interactions. From all these

interactions at Ala 230, Glu 228, Glu 234, Arg 4 are important for therapeutic effects. Many studies have been conducted replacing these amino acid residues with other residues to identify their importance and effects (Lippa et al. 2008; Kashyap et al 2013; Blake, Spencer, Xiao et. al 2010).



(a)



(b)

Figure 15: The binding energy interactions between (a) (2S)-7, 4'-dihydroxy flavan-(4α-8)-epiafzelechin (ligand 6) (b) 4-dodecyl-1, 3, 8-trihydroxy anthraquinone (ligand 51) and PI3 kinase domain

4.3.3 Protein – Ligand binding energy interactions of PI3K (3S2A)

The standard inhibitor chosen for PI3K was Wortmannin (CID_312145). Many ligands from the docked library scored better than the standard inhibitor and provided considerably fine binding energy values.

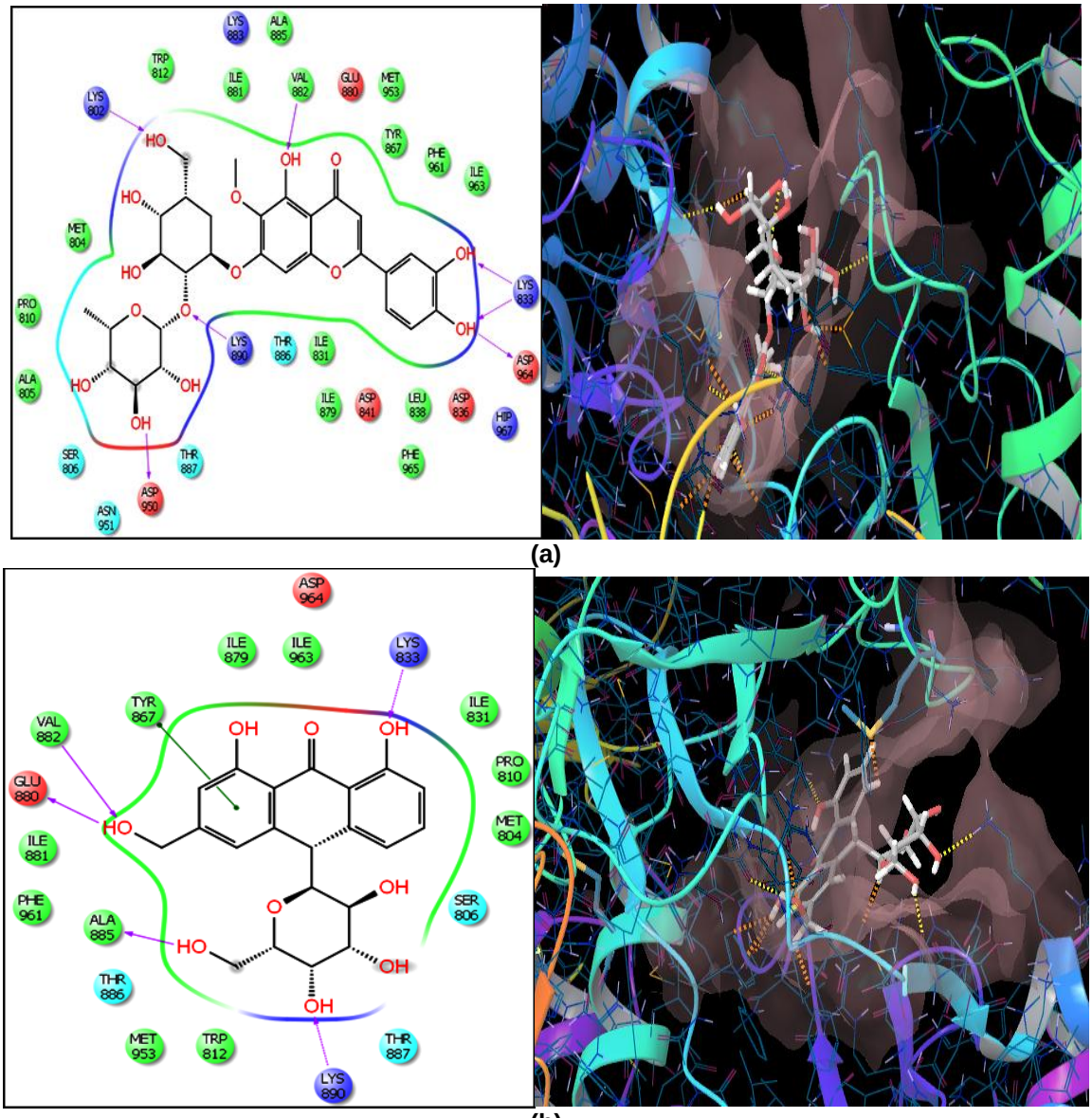
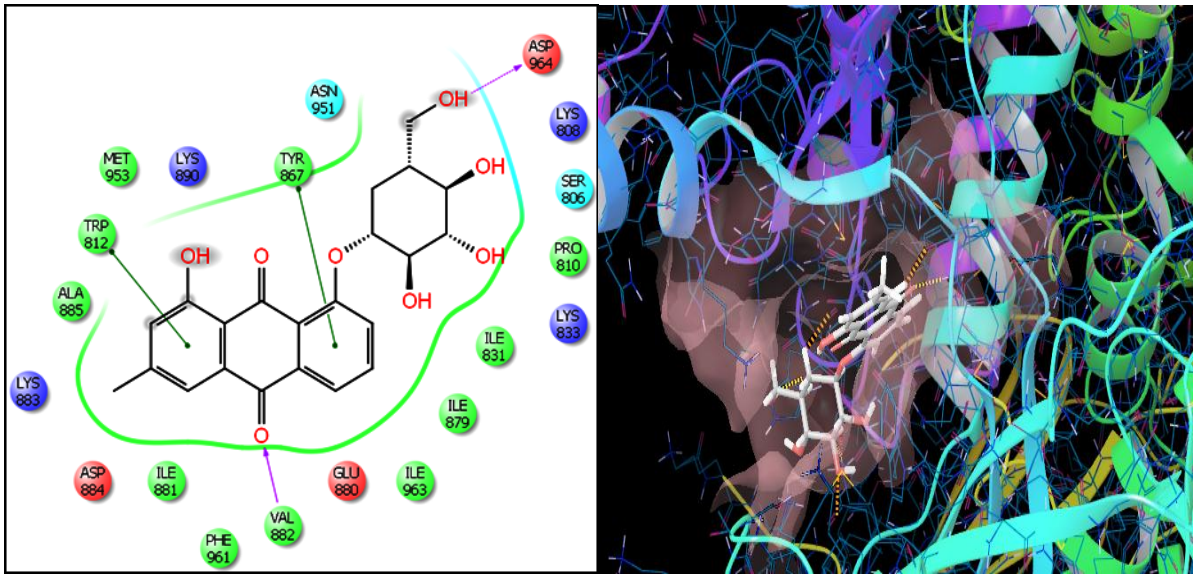


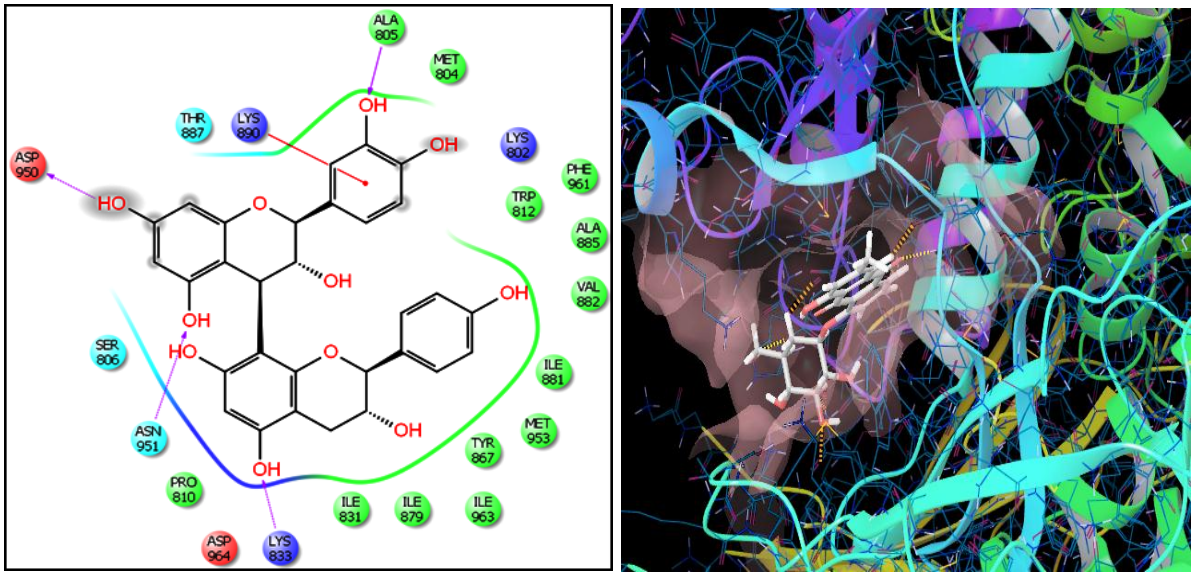
Figure 16: **The binding energy interactions between (a) 5,3,4-tri-hydroxy-6-methoxy-7-O-α-L-rhamnopyranosyl-(1→2)-O-β-D-galactopyranoside (ligand 26) (b) Aloin (ligand 39) and PI3 kinase domain**

The moieties with top scoring binding energy values were (2S)-7,4'-dihydroxyflavan-(4α-8)-epiafzelechin (ligand **6**) (-89.7538 kcal/mol) (Figure 15 a), 4-dodecyl-1,3,8-trihydroxyanthraquinone (ligand **51**) (-87.5229 kcal/mol) (Figure 15 b), ligand **26** (-87.1051 kcal/mol) (Figure 16a), Aloin (ligand **39**) (-81.9574 kcal/mol) (Figure 16b), Chrysophanien (ligand **56**) (-86.1933 kcal/mol) (Figure 17a), Epicatechin-(4β-8)-epicatechin (ligand **4**) (-82.214 kcal/mol) (Figure 17b) (Table 6). On the other hand, Wortmannin had the binding energy score of -79.0654kcal/mol (Figure 17c). Among all

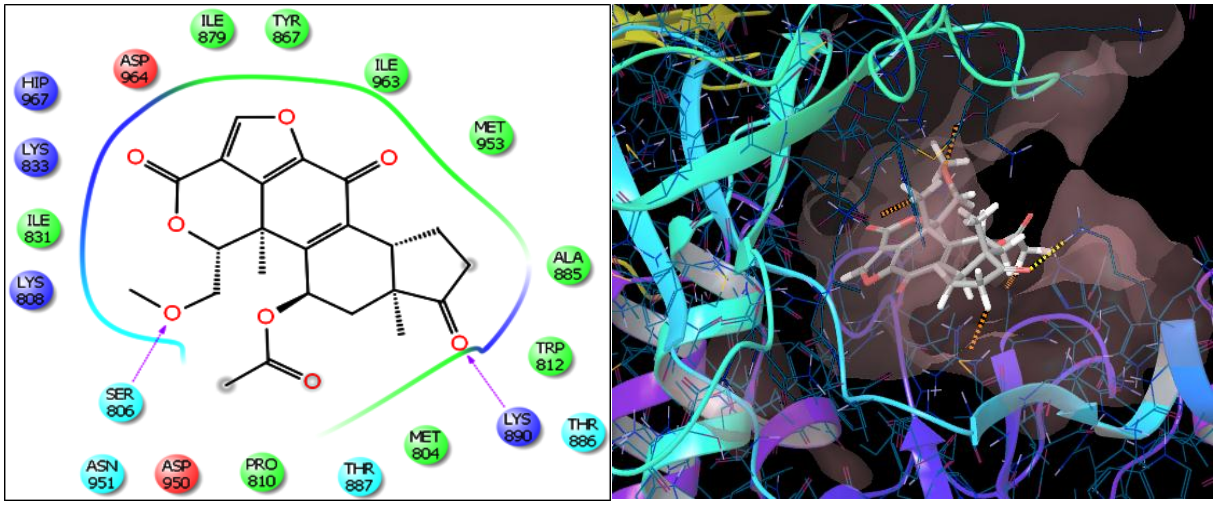
the top scoring molecules ligand **6** is observed to have best binding energy. The binding interactions of this moiety is monitored to be comprised of noticeable coulomb forces, indicating presence of interactions between charged molecules, ions from the moiety and the ions and molecules present on the receptor grid. This further could be confirmed by the presence of π-cation interactions between the Lysine 890 and aromatic ring of the structure as shown above. This moiety has scored (-50.7257 kcal/mol) (Table 6) far better in hydrophobic interactions as compared to Wortmannin (-44.6626 kcal/mol), representing its lipophilicity.



(a)



(b)



(c)

Figure 17: The binding energy interactions between (a) Chrysophanein (ligand 56) (b) Epicatechin (ligand 46) and PI3 kinase domain and (c) Wortmannin.

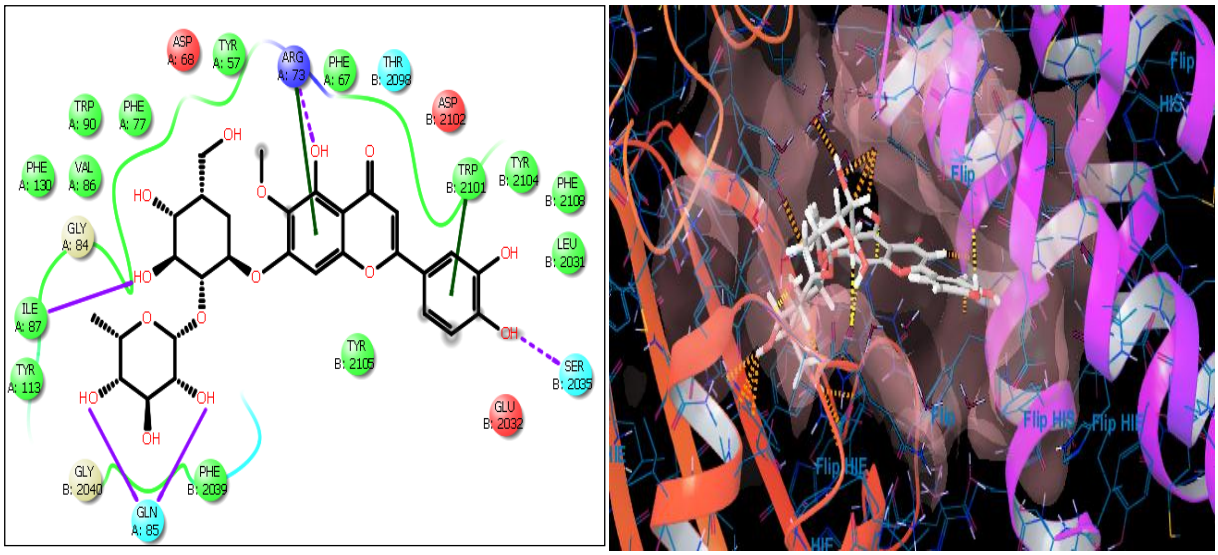
This can again be confirmed on the basis of π- π stacking interactions and π-cation interactions between the ligand and the amino acids present around the Lys 890, Trp 812, Tyr 867 and aromatic rings of ligand on the receptor grid. It is having van der Waals binding energy of -44.7395kcal/mol. Therefore, from these observations we can here state that from all the ligands docked on the PI3 Kinase receptor Ligand **6** indicating the appreciable Non-Covalent interactions which has been observed on the basis of its Coulomb forces, van der Waals forces, and lipophillic interactions etc. ligand **51** has huge hydrophobic interactions. This is so due to presence of large alkylic chain and π-π stacking interactions between aromatic rings of the moiety and amino acids on the receptor grid. As per the ligand receptor Figure, it can be easily observed that Val 882, Ile 879, 831, 963, 881, Met 804, 953, Phe 961, Trp 812, Ala 885,805, Tyr 867, Pro 810 are the amino acids that are involved in the hydrophobic interactions while Trp 812 is also π-π stacking interactions. In contrast to Ligand **51**; ligand **26** is offering a better and comparatively balanced energy distribution in non-covalent interactions. It has provided H-binding energy value (-4.02707 kcal/mol) better than not only the standard inhibitor of PI3 Kinase “Wortmannin” but also then the other two ligands with best binding energy scores. It is showing noteworthy columbic interactions and van der Waals interactions.

PI3K kinase is the major bond in the downstream signalling pathway. Noteworthy interactions have been obtained in the PI3 kinase protein and ligand binding interactions. As per the observations made on the basis of the results obtained from MMGBSA studies, the (2S)-7,4'-dihydroxyflavan-(4α-8)-epiafzelechin (Ligand **6**) (dG Bind score -89.75 kcal/mol) and ligand **26** (dG Bind –87.10kcal/mol) (Table 6) are showing noteworthy interactions. In the ribose pocket (present near Lys 833) the ligand **6** is showing H-back bonding along with covalent interactions with Lys 833 with its di-hydroxyflavan ring and ligand **26** is showing dually H-back bonding interaction with its di-Hydroxy phenyl ring of the moiety additionally; while wortmannin binds covalently to this amino acid residue without any H-bonding (Nishimura, Seigemund et al. 2011; Williams, Hawkins, Walker et al. 2000). This interaction may favor the affinity of binding of these molecules at the receptor pocket. In the affinity binding pocket that is present opposite to Ribose binding pocket (present near Tyr 867 and Ile 879); the ligand **6** is showing efficient $\pi-\pi$ stacking interactions with Tyr 867 which is poor in wortmannin and causes reduction in its efficiency. The ATP binding pocket (cleft between the N-lobe and C-lobe) Ligand **6** is interacting with Val 882 by H-bonding through hydroxyl phenyl residue of the moiety. Same phenyl residue is interacting with Trp 812 with $\pi-\pi$ stacking of molecules which will bring the moieties in opposite directions (Nishimura, Seigemund et al. 2011; Williams, Hawkins, Walker et al. 2000). This might help the moiety to avoid hinderance due to steric clashes of the residues and molecules with Phe 961 which is the reason of disturbance in interactions in wortmannin giving deleterious effect on its affinity for protein. Similar interactions with Val 882 have been observed in ligand **26** although it is lacking the direct interactions with Tyr 867. It can be concluded from the above discussion that ligand **6** and ligand **26** appears to show better interaction with PI3 kinase (Nishimura, Seigemund et al. 2011; Williams, Hawkins, Walker et al. 2000). Their pharmacokinetic profile also has provided satisfactory results.

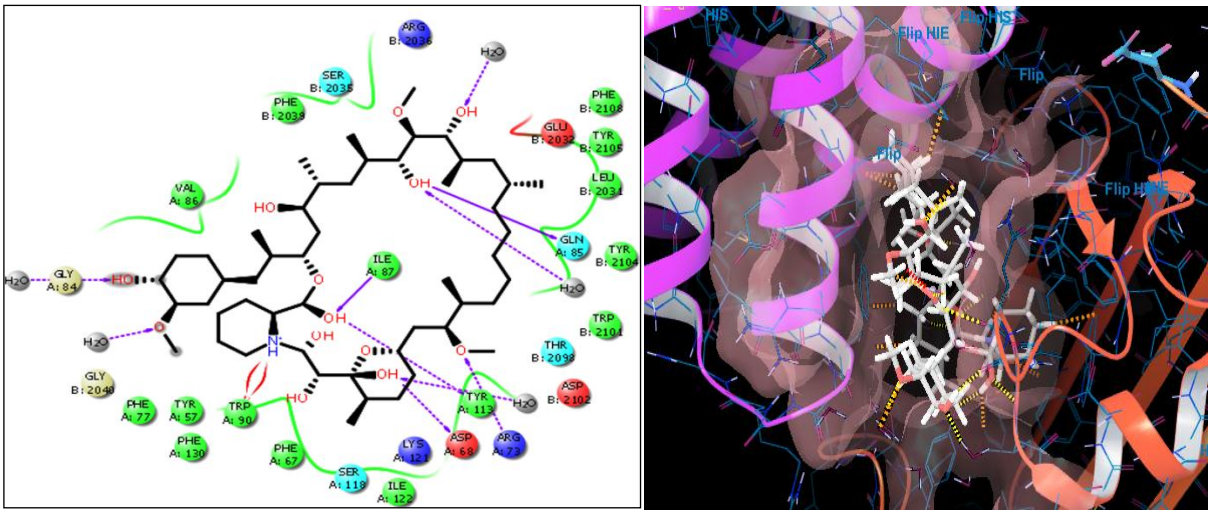
4.3.4. Protein-Ligand binding energy interactions of mTOR (4DRI)

4DRI is the crystal structure of mTOR with FRB domain; that is FKBP rapamycin binding domain. The protein was prepared keeping water molecules intact within the range of 5Å of the receptor grid pocket due to reported interactions of ligands with H_2O

near the receptor pocket. The sirolimus (dG bind score -192.61 kcal/mol) (Table 6) is interacting with Phe 2108, Leu 2031, Tyr 2105, Trp 2101 through vander Waals forces interaction in the hydrophobic pocket of chain B (FRB domain) of protein (Figure 18b). It is also interacting with Ser 2035, Phe 2039 in FRB domain through similar non-covalent interactions. Apart from these necessary interactions it has shown H-bonding with Ile 87 from chain A (FKBP 51 large family domain) of mTOR protein. It has formed H-back bonding with water molecules present in adjacent sites, and also with Asp 68, Arg 73. Ligand **33** and ligand **26** (Figure 18a, b) has illustrated the binding energy score of (-86.226 kcal/mol) and (-81.963 kcal/mol) (Table 6). Ligand **26** is demonstrating stable π-π stacking, non-covalent interactions with Arg 73, along with side chain H-bonding with Arg 73 and Ile 87 in the hydrophobic pocket of FKBP 51 domain (chain A). In the FKBP 51 (chain A) – Ligand **26** complex Arg 73 is interacting via π–π stacking with aryl ring of ligand, while Gln 85 makes H-bonding with its rhamnopyranosyl moiety. With FRB domain (Chain B) it is making π–π stacking interactions with Trp 2104, showing H-back bonding with Ser 2035 and also gives the idea of interactions with Tyr 2108, Leu 2031, Thr 2098 and Phe 2039.



(a)



(b)

Figure 18: The binding energy interactions between (a) 5,3,4-tri-hydroxy-6-methoxy-7-O-α-L-rhamnopyranosyl-(1→2)-O-β-D-galactopyranoside (ligand 26); mTOR inhibitor (b) Sirolimus and mTOR's FRB domain.

The mTOR is downstream signaling path of serine threonine kinase, having important role in various cellular functions. The mTOR is a dimer protein; consisting of Chain A and Chain B, where chain A is FK506 binding protein 51 (FKBP 51) a part of large family of FKB Protein. Sirolimus is believed to form a complex with FK506 binding protein (Chain A) with high affinity and thus forms an activating complex; this complex then bins to Chain B that is FRB's PPIase domain of mTOR protein. The resulting ternary complex then induces activation of various cytoplasmic signaling systems. As appearing in the current interactions, the initial binding with FKBP takes place with the help of amino acid residues Arg 73, Trp 90, Asp 68 and Ile 87. Further; interactions with FRB domain takes place between the C-16 to C-20 of the Sirolimus and amino acid residues Trp 2101, Phe 2108, Thr 2098, Phe 2039 and Ser 2035. These are the major interactions between the Sirolimus-FKBP complex and FRB's PPIase domain in the hydrophobic pocket and activity of Sirolimus is affected if during mutational changes these amino acids are replaced. Although the binding energy of ligand **26** is not comparable to that of sirolumus but, due to appearance of praiseworthy results in the other three Receptors EGFR, Akt kinase and PI3K; and due to comparable results of Dock score of Sirolimus and ligand **26**; we made the observations on the interactions taking place between this moiety and mTOR receptor. It is making

complex with FKBP domain with the help of Arg 73 by making $\pi - \pi$ stacking interactions and H- back bonding with moiety and H- bonding through galacto pyranosyl and rhamnopyranosyl moiety with Ile 87 and Gln 85 in the hydrophobic pocket. Observations can be compared with the dG bind score of lipophilic interactions which is 50.281kcal/mol (Table 6). In the FRB domain it is forming $\pi - \pi$ stacking interactions with Trp 2101 an amino acid residue crucial for the therapeutic effect. The aryl ring's hydroxy group is combining with Ser 2035 through H- back bonding, is another noteworthy interaction. Presence of rhamnopyranosyl ring near Phe 2039 suggests the possible affinity of moiety for the amino acid residue which is also essential for interaction with receptor. This moiety could be further explored for the studying the interactions through molecular dynamics. On the basis of observation made on both moieties that is standard Rapamycin and ligand **26**, it can be observed from their structures that rapamycin is a large moiety with macrolide lactone ring structures in it. Due to which, this moiety could be non-specific and could be easily fit into many other receptors binding cavities and thus could bind to several receptor site also. As a result it shows large number of unwanted toxic side-effects. On the other hand, ligand **26** is a simple yet effective glycoside moiety showing optimum binding energy interactions with mTOR. The mechanism of action of both molecules is different, and there is presence of more H-bonding interactions can be observed in case of ligand **26** binding interactions with mTOR which means efficient and strong interactions, also it is able to show essential interactions with those amino acid residues in the receptor domain pocket that are important for activity and overcoming mutational changes. Further observations on this ligand should be explored.

Table 6: Binding energy score and binding interactions details of the standard inhibitors and moieties with the best binding energy scores:

Receptor	Compound	dG Bind kcal/mol	dGBind HBond kcal/mol	dGBind Coulomb kcal/mol	dGBind Lipo kcal/mol	dGBind vdW kcal/mol	Protien ligands interactions
2ITY (EGFR Kinase)	5,3,4-tri-hydroxy-6-methoxy-7-O-α-L-rhamnopyranosyl-(1→2)-O- β-D-galactopyranoside (**Ligand 26**)	-98.7866	-3.97826	-49.0474	-40.5558	-50.8495	Asp 800,Asn 842, Asp855, Met793
	CID_123631* (Gefitinib)	-86.5585	-1.4467	-32.2301	-40.3107	-52.4562	Asp800, Met 793
3MV5 (Akt kinase)	5,3,4-tri-hydroxy-6-methoxy-7-O-α-L-rhamnopyranosyl-(1→2)-O- β-D-galactopyranoside (Ligand **26**)	-87.3524	-3.92802	-22.9386	-50.6018	-64.1094	Ala A:230,Glu A:228, Thr C:5, ArgC:4, Ser C:7, Gly A: 294
	CID_25227436* (AZD5363)	-76.5959	-3.30357	-70.3947	-34.8066	-39.47	Lys A:179, Arg C:4, Glu A:234, Asp A:292, Glu A: 228, Ala A: 230
3S2A (PI3K kinase)	(2S)-7,4'-dihydroxyflavan-(4α-8)-epiafzelechin (Ligand **6**)	-89.7538	-1.96092	-26.771	-50.7257	-44.7395	Lys 833,Lys, 890,Ala 805, Trp 812, Tyr 867, Val 882
	4-dodecyl-1,3,8-Tri-hydroxyanthraquinone (Ligand **51**)	-87.5229	-0.89798	-24.4046	-62.2153	-41.1089	Trp 812, Ala 885, Val 882
	5,3,4-tri-hydroxy-6-methoxy-7-O-α-L-rhamnopyranosyl-(1→2)-O- β-D-galactopyranoside (Ligand **26**)	-87.1051	-4.02707	-38.455	-53.688	-40.4209	Lys 802, Val 882, Lys 833, Asp 964, Lys 890, Asp 950
	Chrysophanien (Ligand **56**)	-86.1933	-2.03164	-29.0616	-41.0402	-43.3493	Trp 812, Asp 964, Tyr 867, Val 882
	Epicatechin-(4β-8)-epicatechin (Ligand **4**)	-82.214	-2.26407	-35.0477	-51.6668	-45.9068	Ala 805, Lys 890, Asp 950, Asn 951, Lys 833
	Aloin (Ligand **46**)	-81.9574	-2.72678	-41.1386	-47.1693	-35.1176	Lys 890, Ala 885, Val 882, Glu 880, Lys 833, Tyr 867
	CID_312145* (Wortmannin)	-79.0654	-1.78974	-7.87508	-44.6626	-44.3115	Lys 890, Ser 806
4DRI (mTOR)	5,3,4-tri-hydroxy-6-methoxy-7-O-α-L-rhamnopyranosyl-(1→2)-O- β-D-galactopyranoside (Ligand **26**)	-81.964	-2.249	-25.346	-50.281	-46.874	Arg 73, Gln 85,Asp 68, Phe 2108, 2039, Trp 2101, Ser 2305, Thr 2098
	CID 5284616 (Sirolimus)	-192.354	-3.4441	-42.9593	-107.305	-93.642	Ile 87, Trp 2101, Tyr 2105, Arg 73, Gln 85

Table 7: Advanced details of pharmacokinetic properties about the ligands with best binding scores to study their compliance and acceptability parameters as a drug or pharmacophore:

S.no	Compounds	Q P log P o/w (-2.0-6.5)	Q P log HERG (Acceptable range: above -5.0)	QPP Caco (nm/sec) <25-poor >500-great	Q P log BB (-3 to 1.2)	QPP MDCK (nm/sec) <25-poor >500-great	Q P log Kp (-8.0 to -0.1)	Q P log Khsa (Acceptable Range -1.5 to 1.5)
1.	5,3,4-tri-hydroxy-6-methoxy-7-O-α-L-rhamnopyranosyl-(1→2)-O-β-D-galactopyranoside (Ligand **26**)	-1.011	-5.868	3.614	-4.272	1.135	-6.139	-1.043
2.	(2S)-7,4'-dihydroxyflavan-(4b-8)-epiafzelechin (Ligand **6**)	3.417	-7.15	25.435	-2.771	9.35	-4.426	0.646
3.	4-dodecyl-1,3,8-trihydroxyanthraquinone (Ligand **51**)	5.545	-6.365	161.661	-2.466	69.016	-2.97	1.14
4.	Chrysophanein (Ligand **56**)	0.28	-5.333	27.482	-2.592	10.166	-5.064	-0.527
5.	Epicatechin-(4β-8)-epicatechin (Ligand **4**)	0.924	-5.816	2.004	-3.797	0.6	-6.765	-0.044
6.	Aloin (Ligand **39**)	-0.53	-4.478	12.138	-2.724	4.203	-5.769	-0.658
7.	CID 123631 (Iressa)	10.748	-6.769	629.347	0.073	1326.385	-3.2	0.355
8.	CID 312145 (Wortmannin)	13.212	-3.437	-1.21	-1.153	72.057	-4.458	-1.091
9.	CID 25227436 (AZD 5363)	19.155	-5.273	34.261	-1.141	50.117	-5.32	-0.186
10.	CID 5284616 (Sirolimus)	3.88	-5.403	20.892	-3.694	8.362	-3.77	-0.384

4.3 ADMET studies

Pharmacokinetics and pharmacodynamics both studies on moieties are of equal importance. To make search of multi-targeting model more specific; ADME properties of these moieties were also calculated. These properties were examined using Qikprop application of Maestro 9.6. It considers many descriptors to judge the ADME and toxicity properties of molecules. Some descriptors of major importance are given in

table. Ligand **26** has shown partition coefficient values within its range which means that it will have acceptable cell permeability in body and thus would be able to cross the cell membranes for useful pharmacological effect. On the basis of log Kp and log Khsa values (Table 7) it can be stated here that it may show good skin permeability and will be able to bind to the human serum albumin, which shows the presence of hydrophilic groups in moiety. This shows that it will be slowly metabolised in the body and thus, long lasting effect will be there. Although the modification will be required in moiety in order to improve its Caco-2 cell permeability and MDCK cell lines permeability. Such that, the lipophilicity of the molecule could be improved to help it in crossing the Blood brain barrier. Ligand **51** can be considered as a good drug candidate as majority of its qikprop descriptors (Table 7) are indicating values within the required range. Like its partition coefficient value is 3.417 and 5.545 that is considered as to be in the 'sweet' range of Po/w values. Similarly, for Caco-2 cell lines it is matching at the marginal value for ligand 6 and offering noteworthy value for ligand **51** (161.661nm/sec) (Table 7) while the log Kp and Khsa value are lying within the acceptable limit which means these moieties have good cell permeability, lipophilicity, and skin permeability. It can be considered as a molecule with good amalgamation of different properties required to be a good drug candidate. Ligand **51**, ligand **56** and Aloin are two anthraquinone moieties that have provided appreciative binding energy scores with PI3K receptor. These are again signifying proficient ADME properties. Along with Caco-2, BB, Kp and Khsa values in the acceptable range.

CHAPTER – 5

CONCLUSION AND SUMMARY

Chapter - 5

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Cassia fistula contains many chemical components having important pharmacological properties. Noteworthy secondary metabolites includes anthraquinones, anthocyanindins, proanthocyanidins, flavanoids, polyphenols, alkaloids, saponins, coumarins, taninns, fatty acids, sterols, glycosides, proteins, carbohydrates and amino acids. Cytotoxic studies performed on cell lines MCF-7, A-549, and H-460 using various extracts to study their cytotoxic activity. Overall activity in all cell lines appeared noteworthy in hydro-methanolic extract; particularly around the concentration of 50μg/ml. This indicates that polar compounds of Cassia fistula might possess more active anti-proliferative activity. Overall study indicate that the extract hydro-methanolic have shown IC50 value of 38μg/ml for MCF-7, <10 μg/ml for A549 and 42μg/ml for H460 respectively are in agreement with the criteria provided by NCI for extract (as discussed in the Result and discussion section). Consistent results were obtained at different dose concentrations in all three cell lines i.e., A549, H460 and MCF7 preferably at higher concentrations least errors were observed. Although non-metastatic cell line A-549 has shown distinctively noteworthy results with all three extracts. But Hydro-methanolic extract has shown observable results on the basis of in case of other two (H460 and MCF-7) cell lines which have metastatic property; this effect could be gross due to multiple numbers of molecules present in the extract or due to the presence of very few major potent moieties. It can be hypothesized (which should be further experimentally investigated) from the above data that this extract could be effective against the metastatic behavior of cancer cells. Moreover all the extracts were stored at ambient temperature (25-30^{o}C) on shelf in DMSO solvent during experimentation, even when stored for 30 days and used later for MCF-7 cell lines the results were still consistent and comparable which shows the stability of extract in DMSO. Apart from this information gained from the above in vitro cytotoxicity study, we have also found from the in silico studies; that the candidate molecules discussed above with best binding energy score are showing effective protein-ligand interactions along with acceptable pharmacokinetic profile. 5,3,4-tri-hydroxy-6-methoxy-7-O-α-L-rhamnopyranosyl-(1→2)-O-β-D-galactopyranoside (ligand **26**) has

been came out as a common moiety in all the four receptor (namely EGFR, PI3K, Akt and mTOR) with better binding energy score than the standard inhibitors. The in silico study was performed on the moieties of Cassia fistula that are already reported in different extracts. The molecules particularly 5,3,4-tri-hydroxy-6-methoxy-7-O-α-L-rhamnopyranosyl-(1→2)-O-β-D-galactopyranoside (ligand **26**) (dG Bind score -98.7866kcal/mol) for EGFR in comparison to gefitinib (dG Bind score -86.5585kcal/mol); (dG Bind score -87.3524kcal/mol) for Akt as compared to AZD 5363 (dG Bind score -76.5959); (dG Bind score -87.1051kcal/mol) for PI3K as compared to wortmannin (dG Bind score -79.0654kcal/mol) and for mTOR it has shown the (dG Bind score of -81.964kcal/mol) against sirolimus (dG Bind score -192.354kcal/mol), (2S)-7,4'-dihydroxyflavan-(4β-8)-epiafzelechin (ligand **6**) (dG Bind score -89.7538kcal/mol for PI3K) and Epicatechin-(4β-8)-epicatechin (Ligand **4**) (dG Bind score -82.214kcal/mol for PI3K) that are showing best binding energy score are reported to be present in acetone extract of the plant. While 4-dodecyl-1,3,8-trihydroxyanthraquinone (ligand **51**) (dG Bind score -87.5229kcal/mol for PI3K) and chrysophanein (ligand **56**) (dG Bind score -86.1933kcal/mol for PI3K) has been isolated from the methanolic extracts. Thus it is expected from the in vitro cytotoxic studies that isolation of molecules may be performed from hydromethanolic extract of Cassia fistula; to identify the moieties responsible for inhibitory action. Further studies on these moieties need to be performed to study their relevancy as a pharmaceutical drug for multi-targeting the LC and BC. We have also found from the in silico studies; that the candidate molecules aforementioned with best binding energy score are showing effective protein ligand interactions along with acceptable pharmacokinetic profile. 5,3,4-tri-hydroxy-6-methoxy-7-O-α-L-rhamnopyranosyl-(1→2)-O-β-D-galactopyranoside (ligand **26**) has been came out as a common moiety in all the four receptor with better binding energy score than the standard inhibitors. Further studies on these moieties need to be performed to study their relevancy as a pharmaceutical drug for multi-targeting the LC and BC. Besides, it should be explored to gain information about its activity. The isolation of phytoconstituents from the hydromethanolic extract need to be performed to identify the moieties responsible for the versatile cytotoxic results reported in the current in vitro study.

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APPENDIX A

Protein – Ligand binding interactions data on EGFR kinase receptor

Compound	MMGBSA dG Bind	docking score	MMGBSA dG Bind Hbond	MMGBSA dG Bind Coulomb	MMGBSA dG Bind Lipo	MMGBSA dG Bind vdW
5,3,4-tri-hydroxy-6-methoxy-7-O-α-L-rhamnopyranosyl-(1→2)-O- β -D-galactopyranoside	-98.787	-12.714	-3.978	-49.047	-40.556	-50.85
Procyanidin	-72.367	-10.205	-4.593	-67.905	-35.539	-34.204
Rhamnetin 3-O-gentiobioside	-69.45	-10.094	-2.38	-27.194	-27.468	-31.976
Procyanidin B-2	-59.072	-9.386	-5.518	-40.718	-25.937	-38.099
5,7,3',4'-tetrahydroxy-6-methoxyflavone	-59.847	-9.269	-2.774	-22.164	-26.43	-30.07
Epicatechin-(4ß-8)-epicatechin	-72.981	-9.134	-4.193	-49.923	-30.617	-40.045
kaempferol	-58.452	-8.817	-2.2	-17.954	-24.36	-31.579
7- Methyl Physcion	-63.459	-8.679	-0.743	-13.877	-27.497	-35.231
Citreorosein	-62.154	-8.575	-1.124	-21.58	-24.052	-29.478
Emodin	-57.868	-8.479	-0.851	-15.192	-23.896	-29.584
Epiafzelechin-(4ß-8)-epiafzelechin-(4β-8)-epiafzelechin	-50.369	-8.396	-4.065	-52.959	-35.469	-44.365
Physcion	-50.102	-8.374	-0.509	-20.606	-20.404	-31.589
3,4,7,8,4' pentahydroxyflavan	-45.47	-8.34	-2.973	-24.082	-20.735	-22.277
Aloin	-64.899	-8.316	-2.84	-23.624	-37.472	-34.57
Chrysophanol O-ß-D-glucoside	-65.258	-8.295	-3.1	-24.339	-31.263	-40.358
Fistulic acid	-55.437	-8.245	-0.538	44.133	-26.282	-43.082
Chrysophanic acid	-58.372	-8.163	-0.842	-15.73	-23.874	-28.901
Chrysophanol	-58.474	-8.087	-0.842	-15.758	-23.877	-28.878
Fistucacidin	-37.315	-8.057	-3.089	-21.083	-20.395	-21.475
Epiafzelechin 3-O-ß-D-glucopyranoside	-60.564	-8.024	-3.545	-32.96	-25.34	-34.681
Epiafzelechin-(4ß-8,2ß-0-7)-epiafzelechin-(4ß-8)-afzelechin	-60.372	-8.016	-2.632	-30.325	-27.711	-43.321
4-dodecyl-1,3,8-trihydroxyanthraquinone.1	-75.753	-7.957	-0.836	-15.822	-42.308	-48.471
(-) Catechin.1	-59.927	-7.935	-2.607	-28.295	-29.039	-27.202
Rhein	-43.587	-7.887	-1.306	43.052	-19.921	-33.77
Benzyl-2-hydroxy-3,6-	-51.266	-7.814	-0.551	-8.836	-28.706	-33.768

dimethoxybenzoate						
Procyanidin B-2	-54.693	-7.799	-5.33	-1.286	-25.447	-41.76
Leucopelargonidin	-42.43	-7.792	-2.392	-15.364	-28.146	-27.284
1,4,5-Tri-hydroxyanthraquinone	-53.183	-7.788	-0.855	-16.485	-20.465	-26.429
Epiafzelechin-(4ß-8)-epicatechin.1	-59.978	-7.763	-4.383	-51.699	-25.328	-35.127
Epiafzelechin glucopyranoside.1	-64.007	-7.749	-4.705	-24.741	-35.321	-43.057
Chrysophanien	-66.582	-7.717	-2.129	-9.306	-30.59	-36.907
Catechin	-58.732	-7.591	-2.652	-19.754	-29.833	-30.005
Gefitinib	-64.601	-7.515	-0.914	-11.081	-42.382	-39.717
Gefitinib	-86.559	-7.412	-1.446	-32.23	-40.311	-52.456
Sennindin	-51.249	-7.387	-2.271	34.995	-32.333	-49.744
(-)epicatechin	-61.437	-7.305	-2.554	-18.668	-30.564	-30.827
(2S)-7, 4'-dihydroxyflavan-(4ß-8)-epiafzelechin-epiafzelehin	-57.78	-7.006	-4.638	-3.479	-38.798	-45.919
Citreorosein	-54.573	-6.99	-1.069	19.115	-23.423	-30.899
leucoanthocyanidin-5,4-dihydroxyflavan-3,4-diol	-52.011	-6.972	-2.275	-11.684	-27.007	-31.054
(2S)-7,4'-dihydroxyflavan-(4α-8)-epiafzelechin	-70.119	-6.938	-1.895	-35.837	-31.352	-46.028
11-Acetyl-aloe emodin	-62.486	-6.859	-1.37	-27.803	-27.588	-33.701
(2S)-7,4'-dihydroxyflavan-(4ß-8)-epiafzelechin	-71.543	-6.805	-1.146	-27.995	-38.691	-47.178
(2'S)-7-hydroxy-2-(2'-hydroxypropyl)-furfural	-51.365	-6.767	-1.85	-20.177	-25.705	-22.754
Emodin	-52.846	-6.729	-0.848	22.908	-23.924	-29.752
(2S)-7, 4'-dihydroxyflavan-(4ß-8)-epiafzelechin-epiafzelehin	-71.924	-6.636	-4.046	-47.133	-40.006	-45.097
3-formyl-1-hydroxy-8-methoxy-anthraquinone	-56.187	-6.517	-0.251	-13.021	-22.757	-35.76
Epiafzelechin	-56.819	-6.402	-1.954	-16.658	-29.784	-30.859
Epicatechin-(4ß-8)-epicatechin	-60.215	-6.39	-4.375	-2.294	-33.442	-47.342
Epiafzelechin-(4ß-8)-epiafzelechin	-57.71	-6.369	-3.313	-46.55	-26.586	-36.262
Biochanin A	-57.744	-6.351	-0.921	-9.174	-29.959	-34.771

(2S)-7,4'-dihydroxyflavan-(4α-6)-epiafzelechin.1	-68.225	-6.177	-0.661	-19.691	-39.586	-48.153
2,4-Dihydrobenzaldehyde	-28.521	-6.073	-0.594	-11.514	-10.996	-16.421
Vanillic acid	-29.471	-6.019	-0.531	36.044	-16.416	-16.771
(2'S)-7-hydroxy-5-hydroxymethyl-chromone	-43.482	-5.991	-2.153	-19.962	-21.578	-23.369
Physcion	-42.079	-5.981	-0.852	29.002	-20.291	-29.725
Epiafzelechin-(4ß-8,2ß-0-7)-epiafzelechin-(4ß-8)-afzelechin	-54	-5.913	-2.084	-0.158	-30.331	-43.175
Iscoscopoletin	-46.402	-5.827	-1.551	-14.751	-18.53	-26.675
5-(2-hydroxy phenoxy methyl) furfural	-45.245	-5.682	-0.913	-9.421	-18.995	-29.535
Epiafzelechin-(4ß-8)-epicatechin	-39.538	-5.625	-4.869	8.649	-30.658	-39.19
Epiafzelechin-(4ß-8)-epiafzelechin-(4β-8)-epiafzelechin	-65.909	-5.544	-1.999	4.962	-36.325	-52.317
Epiafzelechin-(4ß-8)-epicatechin	-51.919	-5.504	-3.386	-16.492	-20.385	-32.399
Epicatechin-(4ß-8)-epicatechin	-66.359	-5.474	-4.01	-1.958	-32.475	-46.212
(2S)-7,4'-dihydroxyflavan-(4β-6)-epiafzelechin	-61.909	-5.457	-1.383	-22.617	-31.121	-49.985
Sennoside B	-37.182	-5.217	-3.117	68.579	-23.941	-38.589
Epiafzelechin-(4ß-8,2ß-0-7)-epiafzelechin-(4ß-8)-afzelechin	-51.483	-5.12	-1.989	17.331	-26.8	-43.347
Scopoletin	-46.251	-5.093	-1.094	-15.778	-19.882	-24.331
Rhamnetin 3-O-gentiobioside	-60.494	-5.038	-2.244	-3.165	-28.675	-32.092
2,4-Dihydrobenzaldehyde	-22.33	-4.978	-0.61	27.354	-11.015	-16.683
Xanthones	-50.09	-4.906	-0.241	-7.03	-23.079	-25.58
Epiafzelechin-(4ß-8)-epiafzelechin-(4ß-8)-epiafzelechin	-61.676	-4.805	-2.104	5.742	-37.103	-52.327
Epiafzelechin-(4ß-8)-epiafzelechin	-50.289	-4.711	-2.924	3.534	-25.559	-41.129
(2S)-7,4'-dihydroxyflavan-(4α-8)-epiafzelechin.1	-46.281	-4.681	-1.049	15.453	-39.276	-42.223
Rahmnopyranoside	-61.465	-4.66	-3.383	7.309	-41.885	-50.392

Isovanillin	-21.7	-4.565	-0.998	34.269	-14.013	-18.801
(2S)-7, 4'-dihydroxyflavan-(4ß-8)-epiafzelechin-epiafzelehin	-45.724	-4.092	-2.096	4.409	-27.568	-49.498
(2S)-7,4'-dihydroxyflavan-(4ß-8)-epiafzelechin.1	-45.286	-3.736	-1.752	15.955	-32.927	-41.752
Epiafzelechin-(4ß-8)-epiafzelechin-(4ß-8)-epiafzelechin	-47.376	-3.492	-2.045	41.776	-26.604	-52.071
(2S)-7, 4'-dihydroxyflavan-(4ß-8)-epiafzelechin-epiafzelehin	-48.396	-3.466	-1.934	-4.717	-31.247	-43.499
ß- Sitosterol	-58.052	-3.43	-0.369	-4.145	-58.478	-31.107
Benzyl-2-hydroxy-3,6-dimethoxybenzoate	-52.955	-3.427	-0.005	35.857	-33.002	-35.504
Lupeol	-59.78	-3.237	0	-3.118	-51.137	-35.542
Epiafzelechin-(4ß-8)-epiafzelechin	-54.531	-3.1	-2.828	11.843	-29.639	-44.458
Procyanidin B-2	-42.827	-2.904	-4.062	-1.108	-30.545	-41.736
(2S)-7, 4'-dihydroxyflavan-(4ß-8)-epiafzelechin-epiafzelehin	-39.503	-2.356	-2.108	40.62	-27.158	-49.871
Biochanin A	-43.925	-2.147	-1.067	23.068	-27.221	-20.243
Betulinic acid	-57.991	-2.038	-1.274	36.71	-46.933	-33.491
Sennoside B	-23.522	-1.579	-2.296	123.786	-24.115	-44.122

APPENDIX B

Protein – Ligand binding interaction data on Akt kinase receptor

Compounds	MMGBSA dG Bind	docking score	MMGBSA dG Bind vdW	MMGBSA dG Bind Hbond	MMGBSA dG Bind Coulomb	MMGBSA dG Bind Lipo
5,3,4-tri-hydroxy-6-methoxy-7-O-α-L-rhamnopyranosyl-(1→2)-O- β -D-galactopyranoside	-71.562	-11.268	-55.265	-2.889	-45.194	-49.922
5,3,4-tri-hydroxy-6-methoxy-7-O-α-L-rhamnopyranosyl-(1→2)-O- β -D-galactopyranoside	-87.352	-9.921	-64.109	-3.928	-22.939	-50.602
AZD5363	-76.596	-9.844	-39.47	-3.304	-70.395	-34.807
AZD5363	-58.259	-9.357	-44.403	-2.467	-35.969	-34.722
Procyanidin B-2	-20.791	-8.477	-17.261	-3.477	-47.542	-33.967
Fistucacidin	-54.654	-8.445	-36.053	-1.648	-25.429	-35.799
Procyanidin	-24.279	-8.314	-44.183	-3.073	-38.609	-26.792
Leucopelargonidin	-49.561	-8.021	-30.218	-2.493	-21.336	-37.487
(-)Catechin	-46.499	-7.72	-31.438	-1.098	-24.471	-36.066
Epiafzelechin 3-O-β-D-glucopyranoside	-55.86	-7.713	-50.719	-2.054	-35.32	-36.808
Rhamnetin 3-O-gentiobioside	-60.995	-7.406	-34.477	-2.753	-30.313	-37.091
Scopoletin	-51.096	-7.374	-20.513	-0.757	-14.692	-26.818
(-)Epicatechin.1	-59.93	-7.181	-35.137	-3.058	-27.795	-36.66
Catechin	-50.32	-7.149	-34.614	-1.067	-27.556	-35.142
Epiafzelechin-(4β-8)-epicatechin.1	-20.455	-7.116	-16.239	-2.689	-48.802	-34.288
kaempferol	-48.727	-7.083	-38.833	-1.15	-12.283	-31.373
Chrysophanien	-60.266	-7.041	-47.585	-3.355	-18.672	-32.883
Epiafzelechin glucopyranoside	-71.738	-6.935	-44.716	-2.16	-41.663	-49.698
5-(2-hydroxy phenoxy methyl) furfural	-40.514	-6.856	-28.9	-0.609	-13.204	-28.173
4-dodecyl-1,3,8-trihydroxyanthraquinone	-71.665	-6.812	-54.603	-0.658	-5.673	-53.477
5,7,3',4'-tetrahydroxy-6-methoxyflavone	-65.113	-6.71	-41.712	-1.968	-19.21	-38.925
Isovanillin	-14.889	-6.705	-23.907	-0.937	25.782	-20.403
Procyanidin B-2	-19.293	-6.688	-18.573	-3.757	-30.819	-34.002
11-Acetyl-aloe emodin	-50.165	-6.628	-27.112	-1.36	-23.3	-35.393
Biochanin A	-45.563	-6.392	-36.345	-0.859	-19.259	-29.388

Aloin	-29.779	-6.386	-25.602	-4.459	-33.214	-25.665
Citreorosein	-52.532	-6.382	-38.529	-1.087	-19.774	-25.457
(2'S)-7-hydroxy-2-(2'-hydroxypropyl)-furfural	-64.497	-6.329	-34.411	-2.201	-17.38	-38.511
Epiafzelechin-(4β-8)-epiafzelechin	-19.977	-6.269	-18.843	-2.718	-42.642	-34.44
Chrysophanol O-β-D-glucoside	-61.407	-6.243	-38.883	-3.541	-37.701	-32.987
(2'S)-7-hydroxy-5-hydroxymethyl-chromone	-64.27	-5.965	-34.386	-2.201	-17.391	-38.507
(2S)-7,4'-dihydroxyflavan-(4□-8)-epiafzelechin.1	-23.806	-5.846	-53.878	-1.379	1.025	-46.553
Rhein	-40.602	-5.835	-40.515	-2.284	18.382	-24.404
Procyanidin B-2	-6.61	-5.813	-27.175	-3.987	-25.63	-34.057
3,4,7,8,4' pentahydroxyflavan	-43.074	-5.793	-30.063	-1.567	-29.321	-27.096
Physcion	-51.432	-5.745	-37.148	-0.942	-21.729	-24.445
Epiafzelechin-(4β-8)-epicatechin	-18.37	-5.659	-17.542	-2.989	-30.543	-34.271
Leucoanthocyanidin-5,4-dihydroxyflavan-3,4-diol	-44.566	-5.641	-35.975	-1.691	-12.509	-36.004
1,4,5-Tri-hydroxyanthraquinone	-47.09	-5.615	-39.076	-0.377	-9.846	-24.669
Emodin	-49.082	-5.55	-37.307	-0.53	-15.246	-25.612
Benzyl-2-hydroxy-3,6-dimethoxybenzoate	-42.255	-5.257	-34.308	-0.246	-2.066	-37.836
2,4-Dihydrobenzaldehyde	-35.59	-5.214	-23.544	-1.193	-15.586	-13.78
(2S)-7,4'-dihydroxyflavan-(4a-8)-epiafzelechin	-41.247	-5.162	-50.474	-1.27	-19.828	-36.162
Chrysophanol	-47.052	-5.069	-38.602	-0.341	-9.064	-25.99
Epiafzelechin-(4β-8)-epiafzelechin	-18.051	-5.061	-23.936	-2.684	-35.704	-34.413
Chrysophanic acid	-46.705	-5.023	-38.607	-0.341	-9.065	-25.992
Fistulic acid	-41.646	-4.984	-39.927	-2.691	0.729	-18.694
3-formyl-1-hydroxy-8-methoxy-anthraquinone	-46.308	-4.97	-35.839	-1.609	-14.721	-28.557
Epiafzelechin-(4β-8)-epicatechin	-3.155	-4.734	-17.878	-3.68	-22.194	-34.111
Physcion	-36.954	-4.61	-38.125	-1.665	10.123	-24.312

Epiafzelechin	-49.941	-4.512	-34.84	-1.835	-23.913	-36.167
Vanillic acid	-10.237	-4.466	-22.825	-0.479	25.386	-20.453
Iscoscopoletin	-43.225	-4.436	-26.949	-1.212	-9.596	-23.398
(2S)-7,4'-dihydroxyflavan-(4α-6)-epiafzelechin	-44.681	-4.378	-57.873	-0.663	-22.98	-39.363
7- Methyl Physcion	-41.611	-4.293	-41.987	-0.456	-13.494	-18.384
β-Sitosterol	-23.001	-4.159	-9.664	-0.216	-4.543	-77.813
Epiafzelechin-(4β-8)-epiafzelechin	-4.182	-4.139	-17.532	-3.87	-20.844	-33.907
2,4-Dihydrobenzaldehyde	-7.078	-4.05	-22.222	-0.622	20.525	-13.792
Xanthones	-44.035	-3.498	-32.572	-0.001	0.507	-27.704
Citreorosein	-23.115	-2.845	-26.331	-2.496	-0.021	-17.849
Rhamnetin 3-O-gentiobioside	-34.468	-2.773	-41.916	-0.59	-2.236	-28.187
Emodin	-16.267	-1.942	-30.461	-1.022	5.937	-18.199
Biochanin A	-31.166	-1.483	-35.588	-1.372	9.281	-27.45
Lupeol	-8.829	-1.244	-14.483	-0.075	1.319	-64.494
5 (2S)-7,4'-dihydroxyflavan-(4β-8)-epiafzelechin.1	-20.929	1.215	-38.775	-1.922	-21.983	-30.932
Benzyl-2-hydroxy-3,6-dimethoxybenzoate.1	-23.742	1.271	-43.902	-0.007	4.925	-38.5

APPENDIX C

Protein – Ligand binding energy interaction data on PI3K receptor

Compound	MMGBSA dG Bind	MMGBSA dG Bind vdW	docking score	MMGBSA dG Bind Hbond	MMGBSA dG Bind Coulomb	MMGBSA dG Bind Lipo
5,3,4-tri-hydroxy-6-methoxy-7-O-α-L-rhamnopyranosyl-(1→2)-O- β -D-galactopyranoside	-87.105	-40.421	-12.918	-4.027	-38.455	-53.688
Sennindin	-77.604	-40.343	-12.231	-5.136	-17.879	-46.193
Chrysophanol O-□-D-glucoside	-78.284	-44.627	-11.338	-1.098	-16.975	-39.569
Fistucacidin.	-63.348	-34.125	-11.251	-0.965	-22.903	-40.156
Chrysophanien	-86.193	-43.349	-11.087	-2.032	-29.062	-41.04
5,7,3',4'-tetrahydroxy-6-methoxyflavone	-66.178	-37.958	-10.71	-1.735	-15.367	-36.771
Rhein	-57.881	-30.844	-10.414	-3.612	-5.014	-24.534
Procyanidin	-58.089	-39.292	-10.263	-3.224	-35.719	-44.373
Methyl Physcion	-65.734	-37.139	-10.109	-1.194	-18.693	-32.086
Aloin	-81.957	-35.118	-10.014	-2.727	-41.139	-47.169
3,4,7,8,4' pentahydroxyflavan	-67.425	-29.322	-9.68	-0.839	-28.334	-40.722
Rhamnetin 3-O-gentiobioside	-63.607	-40.231	-9.537	-1.683	-21.917	-35.844
Chrysophanol	-53.882	-30.687	-9.509	-0.748	-16.677	-26.225
Chrysophanic acid	-53.915	-30.676	-9.451	-0.748	-16.691	-26.231
Leucoanthocyanidin-5,4-dihydroxyflavan-3,4-diol	-60.781	-33.469	-9.44	-1.277	-20.773	-39.742
Catechin	-60.671	-32.839	-9.402	-1.393	-26.708	-40.461
Physcion	-62.366	-32.289	-9.359	-1.343	-24.609	-24.152
11-Acetyl-aloe emodin	-65.824	-34.847	-9.273	-0.872	-21.442	-35.959
Epicatechin-(4β-8)-epicatechin	-82.214	-45.907	-9.208	-2.264	-35.048	-51.667
Leucopelargonidin	-58.136	-29.395	-9.151	-1.873	-30.898	-35.192
Kaempferol	-62.412	-32.5	-9.072	-2.637	-27.311	-30.287
Procyanidin B	-61.067	-39.965	-9.06	-3.164	-35.454	-43.599
Physcion	-61.279	-31.355	-9.011	-2.976	-20.163	-23.955
Rahmnopyranoside	-80.59	-50.374	-8.977	-4.132	-45.408	-43.297
4-dodecyl-1,3,8-trihydroxyanthraquinone.	-87.523	-41.109	-8.678	-0.898	-24.405	-62.215
Epiafzelechin-(4β-8)-epicatechin	-78.363	-40.202	-8.661	-2.685	-45.681	-41.76
Epicatechin	-62.125	-35.391	-8.617	-1.295	-16.444	-39.335
1,4,5-Tri-hydroxyanthraquinone	-56.123	-30.842	-8.539	-0.76	-20.263	-23.952
Emodin	-63.962	-34.412	-8.413	-0.963	-19.539	-28.544
3-formyl-1-hydroxy-8-methoxy-anthraquinone	-60.135	-33.797	-8.413	-0.423	-8.698	-28.607
Citreorosein	-62.522	-35.952	-8.391	-0.965	-19.556	-28.612
Epiafzelechin 3-O-β-D-glucopyranoside	-66.832	-36.563	-8.301	-1.273	-23.578	-39.073
5-(2-hydroxy phenoxy methyl) furfural	-52.562	-27.593	-8.235	-0.814	-13.976	-26.859

(2S)-7,4'-dihydroxyflavan-(4β-8)-epiafzelechin	-81.018	-52.778	-8.076	-2.311	-27.866	-52.021
(2'S)-7-hydroxy-2-(2'-hydroxypropyl)-furfural	-69.233	-31.373	-8.035	-0.468	-13.934	-39.585
Catechin	-59.957	-36.518	-7.955	-1.498	-24.412	-39.569
Scopoletin	-58.258	-28.238	-7.936	-0.787	-16.712	-26.553
(2'S)-7-hydroxy-5-hydroxymethyl-chromone	-66.891	-32.929	-7.914	-0.658	-13.96	-38.977
(2S)-7,4'-dihydroxyflavan-(4α-8)-epiafzelechin	-89.754	-44.739	-7.82	-1.961	-26.771	-50.726
Epiafzelechin.	-51.377	-25.575	-7.652	-1.397	-14.01	-40.225
(2S)-7,4'-dihydroxyflavan-(4β-6)-epiafzelechin	-71.552	-35.01	-7.467	-2.088	-30.498	-44.351
Fistulic acid	-63.062	-43.065	-7.362	-3.17	-9.234	-31.723
Benzyl-2-hydroxy-3,6-dimethoxybenzoate	-55.145	-35.445	-7.348	-0.291	-1.512	-34.976
Citreorosein	-43.648	-36.12	-7.287	-1.404	21.625	-29.428
(2S)-7, 4'-dihydroxyflavan-(4β-8)-epiafzelechin-epiafzelehin	-53.372	-32.549	-7.237	-4.652	-52.04	-31.295
Epiafzelechin-(4β-8)-epiafzelechin	-49.814	-28.668	-7.235	-1.711	-26.448	-43.388
2,4-Dihydrobenzaldehyde	-36.612	-19.218	-7.175	-0.709	-16.519	-14.928
Epicatechin-(4β-8)-epicatechin	-55.012	-40.045	-7.166	-2.248	-13.119	-49.184
Epiafzelechin-(4β-8)-epiafzelechin-(4β-8)-epiafzelechin	-40.282	-32.359	-7.119	-5.753	-26.185	-37.857
Epiafzelechin-(4β-8)-epicatechin	-62.059	-38.628	-7.11	-2.485	-18.025	-42.396
Biochanin A	-71.079	-38.384	-7.09	-0.639	-15.104	-38.424
Epiafzelechin glucopyranoside	-59.138	-38.651	-7.088	-3.284	-20.269	-39.222
Procyanidin B	-64.242	-39.14	-7.009	-2.85	-22.159	-43.503
Xanthones	-54.479	-29.488	-6.988	-0.25	-3.348	-29.212
Procyanidin B	-54.942	-28.61	-6.616	-2.841	-29.564	-44.045
Epiafzelechin-(4β-8)-epicatechin	-62.879	-39.396	-6.527	-3.554	-39.974	-43.114
Emodin	-60.557	-34.505	-6.494	-1.979	-8.336	-28.265
(2S)-7,4'-dihydroxyflavan-(4α-8)-epiafzelechin.1	-89.063	-45.305	-6.345	-2.965	-29.014	-50.928
Isovanillin	-29.543	-23.445	-6.32	-0.564	20.355	-20.274
Vanillic acid	-18.431	-25.77	-6.169	-0.905	34.728	-20.186
Iscoscopoletin	-55.545	-27.362	-6.077	-0.551	-16.286	-26.494
Epiafzelechin-(4β-8)-epiafzelechin	-56.659	-37.079	-6.042	-2.249	-11.137	-42.98
2,4-Dihydrobenzaldehyde	-12.353	-21.144	-5.569	-0.599	23.295	-14.703
Wortmannin	-79.065	-44.312	-5.479	-1.79	-7.875	-44.663
Epiafzelechin-(4β-8)-epiafzelechin	-56.246	-39.007	-5.435	-3.314	-29.007	-43.102

(2S)-7, 4'-dihydroxyflavan-(4β-8)-epiafzelechin-epiafzelehin	-46.572	-33.007	-5.398	-3.822	-39.634	-43.155
(2S)-7,4'-dihydroxyflavan-(4α-6)-epiafzelechin	-66.587	-45.026	-5.238	-1.831	-21.17	-38.306
Epiafzelechin-(4β-8)-epiafzelechin-(4β-8)	-34.663	-41.324	-5.21	-2.62	-31.356	-18.104
(2S)-7, 4'-dihydroxyflavan-(4β-8)-epiafzelechin-epiafzelehin	-43.547	-32.124	-5.161	-3.981	-28.976	-42.601
Sennoside B	-39.23	-47.118	-4.831	-1.458	5.263	-27.773
Benzyl-2-hydroxy-3,6-dimethoxybenzoate	-51.066	-30.893	-4.441	-2.746	-2.551	-34.603
Epicatechin-(4β-8)-epicatechin	-70.421	-49.321	-4.274	-2.372	-13.887	-51.97
Betulinic acid	-51.556	-22.739	-3.843	-2.366	6.117	-56.973
β- Sitosterol	-73.203	-33.369	-3.806	-1.425	-7.926	-64.534
Lupeol	-42.824	-2.741	-3.697	-0.221	-9.183	-73.619
Rhamnetin 3-O-gentiobioside	-58.75	-37.776	-2.805	-1.917	-16.725	-34.209
(2S)-7,4'-dihydroxyflavan-(4β-8)-epiafzelechin	-56.771	-49.5	-2.755	-3.165	11.457	-50.438
Epiafzelechin-(4β-8)-epiafzelechin-(4β-8)-epiafzelechin	-9.852	-25.436	-2.712	-4.301	-49.914	-19.888
Biochanin A	-46.14	-35.088	-2.238	-0.298	28.509	-37.354
(2S)-7, 4'-dihydroxyflavan-(4β-8)-epiafzelechin-epiafzelehin	-43.735	-44.721	-2.082	-5.404	-35.361	-38.304
Sennoside B	-55.781	-51.155	-1.843	-3.833	45.993	-47.124
(2S)-7, 4'-dihydroxyflavan-(4β-8)-epiafzelechin-epiafzelehin	-19.969	-15.724	-0.265	-4.03	-20.104	-42.558

APPENDIX D

Protein – Ligand Interaction data of mTOR receptor

Compounds	MMGBSA dG Bind	MMGBSA dG Bind vdW	docking score	MMGBSA dG Bind Hbond	MMGBSA dG Bind Coulomb	MMGBSA dG Bind Lipo
Epiafzelechin-(4β-8)-epiafzelechin-(4β-8)-epiafzelechin	-24.302	-6.334	-16.082	-3.196	-62.217	-55.847
5,3,4-tri-hydroxy-6-methoxy-7-O-α-L-rhamnopyranosyl-(1→2)-O- β -D-galactopyranoside	-81.964	-46.874	-15.204	-2.249	-25.346	-50.281
Procyanidin	-72.715	-44.854	-14.996	-3.827	-54.032	-36.749
(2S)-7, 4'-dihydroxyflavan-(4β-8)-epiafzelechin-epiafzelehin	-39.114	-45.061	-14.46	-1.822	-42.421	-45.873
(2S)-7, 4'-dihydroxyflavan-(4β-8)-epiafzelechin-epiafzelehin	-18.424	-52.101	-13.032	-1.712	-3.363	-42.864
Procyanidin B-2	-60.972	-50.045	-12.987	-3.073	-36.612	-36.306
Epiafzelechin-(4β–8)-epicatechin	-65.529	-47.088	-12.673	-0.958	-37.692	-37.086
(2S)-7, 4'-dihydroxyflavan-(4β-8)-epiafzelechin-epiafzelehin	-22.917	-49.78	-12.281	-1.69	-10.618	-45.86
Epiafzelechin glucopyranoside	-69.603	-46.935	-11.951	-2.376	-28.116	-38.841
Epiafzelechin 3-O-β-D-glucopyranoside	-51.484	-39.036	-11.914	-2.673	-22.991	-33.737
Epiafzelechin-(4ß-8)-epiafzelechin	-49.649	-46.142	-11.453	-1.092	-19.443	-37.091
Epicatechin-(4ß-8)-epicatechin	-64.77	-53.581	-11.279	-1.818	-40.115	-39.457
Rahmnopyranoside	-37.899	-59.068	-11.027	-1.713	20.999	-51.57
(2S)-7,4'-dihydroxyflavan-(4ß-8)-epiafzelechin.1	-73.165	-55.704	-10.878	-2.011	-19.693	-43.469
(2S)-7,4'-	-70.125	-46.45	-10.86	-2.538	-41.835	-42.545

dihydroxyflavan-(4α-8)-epiafzelechin.1						
Epiafzelechin-(4ß-8)-epicatechin.1	-33.526	-42.486	-10.833	-1.116	-22.481	-35.971
Aloin	-55.885	-36.262	-10.625	-1.216	-33.154	-30.338
(2S)-7,4'-dihydroxyflavan-(4α-6)-epiafzelechin	-67.454	-52.26	-10.561	-1.588	-23.91	-45.259
Rhamnetin 3-O-gentiobioside	-47.518	-31.829	-10.494	-1.102	-25.34	-22.151
Leucoanthocyanidin-5,4-dihydroxyflavan-3,4-diol	-50.636	-33.048	-10.433	-1.323	-11.179	-29.708
Procyanidin B-2	-39.116	-38.846	-10.392	-1.585	-19.844	-42.101
Chrysophanol O-ß-D-glucoside	-73.685	-47.319	-10.247	-1.127	-33.027	-29.378
Chrysophanien	-58.716	-43.464	-10.077	-1.497	-10.25	-29.867
4-dodecyl-1,3,8-trihydroxyanthraquinone	-77.699	-46.979	-10.051	-0.299	-14.121	-49.424
Epicatechin-(4ß-8)-epicatechin	-47.723	-52.55	-9.908	-1.539	-9.477	-36.768
Sennoside B	-42.724	-53.926	-9.838	-2.311	56.952	-44.662
Epiafzelechin-(4ß-8)-epiafzelechin-(4ß-8)-epiafzelechin	3.86	-32.575	-9.836	-2	-6.23	-59.582
ß-Sitosterol	-86.226	-38.681	-9.723	-0.001	-8.064	-83.348
(2S)-7,4'-dihydroxyflavan-(4α-8)-epiafzelechin	-36.842	-52.461	-9.713	-2.804	0.892	-40.061
(2S)-7,4'-dihydroxyflavan-(4ß-6)-epiafzelechin	-55.65	-48.943	-9.565	-0.872	-19.906	-34.068
Sennindin	-48.282	-44.458	-9.555	-1.017	-6.981	-29.241
Fistucacidin	-41.898	-33.731	-9.503	-2.003	-17.274	-24.715
5,7,3',4'-tetrahydroxy-6-methoxyflavone	-49.933	-32.373	-9.486	-2.822	-23.256	-21.889
Epiafzelechin-(4ß-8)-epicatechin	-40.723	-49.111	-9.344	-1.036	-18.131	-36.904
3,4,7,8,4' pentahydroxyflavan	-48.277	-31.269	-9.185	-1.536	-22.007	-27.627
Procyanidin B-2	-32.464	-53.972	-9.157	-3.132	4.768	-36.178

(2S)-7, 4'-dihydroxyflavan-(4β-8)-epiafzelechin-epiafzelehin	-20.778	-53.603	-9.062	-2.341	-0.714	-43.495
Leucopelargonidin	-45.513	-32.143	-8.975	-1.612	-21.819	-22.995
Kaempferol	-51.988	-32.649	-8.895	-1.722	-22.292	-23.964
Lupeol	-53.071	-37.057	-8.657	-1.035	-4.202	-54.102
(2S)-7,4'-dihydroxyflavan-(4ß-8)-epiafzelechin	-36.669	-52.567	-8.626	-0.688	14.798	-41.461
Epiafzelechin-(4β-8)-epiafzelechin	-38.161	-47.126	-8.599	-1.095	-12.532	-37.633
Catechin	-49.168	-31.285	-8.547	-2.864	-20.638	-21.944
11-Acetyl-aloe emodin	-58.251	-37.71	-8.383	-0.292	-14.602	-29.578
Catechin	-46.606	-30.712	-8.319	-2.751	-17.49	-22.818
1,4,5-Tri-hydroxyanthraquinone	-41.017	-25.064	-8.292	-0.495	-17.122	-16.183
Biochanin A	-50.059	-28.086	-8.167	-0.516	-10.283	-26.918
Epicatechin	-44.302	-32.22	-8.141	-2.406	-16.92	-24.585
(2'S)-7-hydroxy-5-hydroxymethyl-chromone	-57.731	-29.904	-8.128	-1.408	-18.409	-27.053
Methyl Physcion	-51.579	-32.833	-8.022	-0.712	-14.524	-24.251
Rhein	-21.066	-32.536	-7.967	-2.59	14.471	-15.151
(2'S)-7-hydroxy-2-(2'-hydroxypropyl)-furfural	-57.901	-29.571	-7.926	-1.482	-19.905	-27.057
Emodin	-46.234	-31.326	-7.818	-0.671	-17.746	-16.585
Benzyl-2-hydroxy-3,6-dimethoxybenzoate	-47.3	-33.039	-7.726	-1.133	-12.201	-22.882
Citreorosein	-48.635	-31.967	-7.598	-2.168	-18.658	-17.915
Chrysophanic acid	-46.554	-29.402	-7.544	-0.678	-21.049	-15.667
Chrysophanol	-46.554	-29.403	-7.544	-0.678	-21.049	-15.667
Epiafzelechin	-45.826	-31.391	-7.452	-1.986	-15.008	-23.161
Fistulic acid	-39.532	-35.851	-7.387	-2.849	17.386	-20.496
3-formyl-1-hydroxy-8-methoxy-anthraquinone	-54.1	-37.566	-7.372	-0.457	-9.998	-21.025
(2S)-7, 4'-dihydroxyflavan-(4ß-8)-epiafzelechin-epiafzelehin.1	4.091	-47.119	-7.189	-1.768	16.498	-43.105
Epiafzelechin-(4ß-8)-	-13.604	-46.671	-7.136	-2.272	-8.144	-57.11

epiafzelechin-(4ß-8)-epiafzelechin						
Physcion	-36.041	-22.307	-7.119	-0.801	-22.844	-13.228
Xanthones	-44.858	-26.561	-7.085	0	-1.065	-23.267
Rhamnetin 3-O-gentiobioside	-38.563	-31.259	-6.916	-1.882	-14.713	-22.58
5-(2-hydroxyphenoxymethyl)furfural	-42.99	-27.889	-6.841	-0.247	-9.159	-22.898
Epicatechin-(4ß-8)-epicatechin.1	-53.203	-52.077	-6.31	-1.595	-9.782	-38.444
Epiafzelechin-(4ß-8)-epiafzelechin-(4ß-8)-epiafzelechin	30.733	-15.092	-6.058	-2.841	-5.445	-52.978
Scopoletin	-40.143	-22.652	-5.946	-0.22	-4.507	-17.757
Emodin	-33.625	-31.796	-5.926	-0.889	1.397	-17.232
Epiafzelechin-(4ß-8)-epiafzelechin	-37.927	-48.183	-5.911	-2.068	-6.329	-36.237
Iscoscopoletin	-37.913	-26.247	-5.851	-1.211	-9.958	-17.11
Sennoside B	-0.978	-50.421	-5.702	-1.785	45.484	-31.316
Physcion	-23.516	-28.279	-5.512	-1.911	-1.434	-13.258
2,4-Dihydrobenzaldehyde	-27.511	-19.612	-5.294	-0.324	-6.361	-11.897
Benzyl-2-hydroxy-3,6-dimethoxybenzoate	-42.714	-35.652	-4.685	-0.972	16.486	-27.272
Isovanillin	-15.817	-24.417	-4.651	-0.006	37.157	-15.641
Betulinic acid	-37.478	-46.111	-4.194	-1.176	34.612	-55.15
2,4-Dihydrobenzaldehyde	-13.867	-20.173	-4.107	-0.054	21.62	-11.349
Vanillic acid	-14.239	-21.604	-4.102	-1.051	2.684	-13.258
Citreorosein	-29.157	-32.183	-2.473	-1.534	12.456	-17.718
Biochanin A	-32.453	-26.15	-0.964	-1.716	13.842	-22.37

APPENDIX E

Pharmacokinetic properties of Ligands from *Cassia fistula* used to study the Binding energy interactions on various receptors

APPENDIX F

PUBLICATIONS

1. Pharmacological and Chemical potential of Cassia fistula L- A Critical Review (Communicated).
2. Simulated Binding Interaction of Cassia fistula Phytochemicals on Multiple Interlinking Targets of the Kinase Family along with in vitro Cytotoxicity studies (under preparation).

Compound	mol MW (130-725)	dipole	donorHB (0-6)	accptHB (2-20)	QPlogPo/w -2-6.5	QPPCaco (nm/sec) <25% poor >500 great	QPlogBB -3-1.2	QPlogKp -8.0- (-0.1)	QPlogKhsa -1.5-1.5	Percent Human Oral Absorption
Procyanidin B-2	578.528	5.75	10	10.9	0.211	0.937	-4.104	-7.436	-0.231	0
Epiafzelechin-(4β-8)-epiafzelechin	546.529	4.497	8	9.4	1.55	6.688	-3.073	-5.724	0.093	24.874
Epiafzelechin-(4β-8)-epicatechin	562.529	5.215	9	10.15	0.896	2.596	-3.562	-6.545	-0.068	0.731
Epicatechin-(4β-8)-epicatechin	562.529	5.736	9	10.15	0.948	2.029	-3.797	-6.745	-0.039	0
(2S)-7,4'-dihydroxyflavan-(4β-8)-epiafzelechin	514.531	5.34	6	6.95	3.046	28.836	-2.351	-4.57	0.55	44.995
(2S)-7,4'-dihydroxyflavan-(4α-8)-epiafzelechin	514.531	9.321	6	6.95	3.416	25.556	-2.765	-4.423	0.645	46.219
(2S)-7,4'-dihydroxyflavan-(4β-6)-epiafzelechin	514.531	7.671	6	6.95	3.549	37.395	-2.637	-4.034	0.635	49.957
(2S)-7,4'-dihydroxyflavan-(4α-6)-epiafzelechin	514.531	7.902	6	6.95	3.54	33.621	-2.71	-4.131	0.645	49.081
Epiafzelechin-(4β-8)-epiafzelechin-(4β-8)-epiafzelechin	834.786	8.785	13	14.85	1.699	0.273	-5.599	-7.436	0.156	0
(2S)-7, 4'-dihydroxyflavan-(4β-8)-epiafzelechin-epiafzelehin	786.787	9.49	10	11.65	4.111	1.569	-5.064	-5.777	0.931	15.642
Chrysophanol O-β-D-glucoside	416.384	6.293	4	13	-0.469	28.105	-2.503	-5.088	-0.793	50.129
Epiafzelechin 3-O-β-D-glucopyranoside	436.415	4.331	7	13.2	-0.663	19.53	-2.633	-5.077	-0.76	33.207
Epiafzelechin	274.273	4.648	4	4.7	1.148	147.74	-1.38	-3.818	-0.262	72.499
Sennoside B	534.521	4.799	2	7.5	4.221	0.703	-2.505	-5.988	0.553	35.963
Chrysophanic acid	254.242	4.084	0	3.5	1.802	264.166	-0.975	-3.642	-0.128	80.843
Physcion	272.214	5.013	2	5	0.274	24.38	-2.01	-5.562	-0.356	53.372
Citreorosein	286.24	4.255	2	5.95	0.259	24.686	-2.148	-5.521	-0.404	53.383
Epiafzelechin-(4β-8,2β-7)-epiafzelechin-(4β-8)-afzelechin	816.77	6.276	11	14.1	2.215	1.685	-4.08	-6.184	0.327	5.094
Xanthones	196.205	2.978	0	2.5	2.744	3576.469	0.182	-1.122	-0.151	100

5-(2-hydroxy phenoxymethyl) furfural	218.209	4.395	1	4	1.722	592.438	-0.887	-2.455	-0.308	86.656
Epiafzelechin glucopyranoside	434.442	4.522	7	11.5	-0.129	16.534	-2.706	-5.258	-0.583	35.036
Procyanidin	594.528	6.494	10	11.65	0.484	3.532	-3.476	-5.99	-0.336	0.715
(2'S)-7-hydroxy-5-hydroxymethyl-chromone	250.251	4.953	3	6.65	0.35	165.188	-1.483	-3.975	-0.588	68.695
Benzyl-2-hydroxy-3,6-dimethoxybenzoate	288.299	3.903	0	3.25	3.641	1929.349	-0.484	-1.373	0.222	100
1,4,5-Tri-hydroxyanthraquinone	256.214	3.157	0	3.25	1.433	113.592	-1.319	-4.161	-0.218	72.12
(2'S)-7-hydroxy-2-(2'-hydroxypropyl)-furfural	250.251	4.953	3	6.65	0.35	165.188	-1.483	-3.975	-0.588	68.695
Chrysophanol	254.242	4.084	0	3.5	1.802	264.166	-0.975	-3.642	-0.128	80.843
Chrysophanien	414.411	5.695	4	11.3	0.28	27.482	-2.592	-5.064	-0.527	54.344
5,3,4-tri-hydroxy-6-methoxy-7-O-α-L-rhamnopyranosyl-(1→2)-O- β -D-galactopyranoside	622.579	6.244	8	18.85	-1.011	3.614	-4.272	-6.139	-1.043	0
5,7,3',4'-tetrahydroxy-6-methoxyflavone	318.282	6.087	3	5.5	1.227	73.961	-1.809	-4.528	-0.157	67.583
Fistucacidin	290.272	5.838	5	6.4	0.279	63.883	-1.835	-4.438	-0.497	60.892
leucoanthocyanidin-5,4-dihydroxyflavan-3,4-diol	274.273	4.674	4	5.65	1.014	236.625	-1.205	-3.262	-0.37	75.375
3,4,7,8,4' pentahydroxyflavan	290.272	5.28	5	6.4	0.329	70.441	-1.765	-4.357	-0.484	61.945
kaempferol	286.24	7.397	3	4.5	1.192	57.833	-1.895	-4.474	-0.159	65.464
Leucopelargonidin	290.272	3.475	5	6.4	0.31	70.438	-1.789	-4.339	-0.498	61.832
β- Sitosterol	414.713	1.744	1	1.7	7.546	3405.933	-0.347	-1.648	2.043	100
Catechin	290.272	3.257	5	5.45	0.467	52.995	-1.918	-4.699	-0.422	60.542
Fistulic acid	374.303	3.132	1	6.75	1.728	9.872	-2.132	-5.361	-0.319	54.863
3-formyl-1-hydroxy-8-methoxy-anthraquinone	282.252	4.594	0	6.5	0.544	122.524	-1.401	-4.264	-0.764	67.503
Rhein	284.225	2.458	1	5.5	0.961	6.761	-1.948	-5.505	-0.488	47.428
Sennindin	540.482	7.922	3	7.45	2.615	0.336	-3.784	-7.416	0.388	7.874
Aloin	418.399	3.969	5	11.7	-0.53	12.138	-2.724	-5.769	-0.658	30.29
Emodin	270.241	5.476	1	4.25	1.253	80.258	-1.529	-4.701	-0.104	68.368

Scopoletin	192.171	5.318	1	4	0.863	666.095	-0.546	-3.018	-0.483	82.536
Biochanin A	286.284	4.846	1	4	2.474	588.347	-0.763	-2.794	0.113	91.004
11-Acetyl-aloe emodin	328.321	7.781	1	6.9	1.387	73.979	-1.954	-4.357	-0.268	68.522
(-)Catechin	290.272	2.942	5	5.45	0.494	55.168	-1.847	-4.689	-0.405	61.012
Lupeol	426.724	2.19	1	1.7	7.037	4441.7	0.12	-1.919	1.999	100
(-)Epicatechin	290.272	4.628	5	5.45	0.481	53.349	-1.875	-4.707	-0.41	60.671
2,4-Dihydrobenzaldehyde	138.123	1.557	1	2.5	1.181	209.904	-0.976	-4.017	-0.602	75.42
Isovanillin	168.149	3.303	2	3.5	1.048	82.903	-0.847	-3.728	-0.749	67.419
Glyceryl trilinolate	879.398	4.243	0	6	17.653	382.886	-4.801	0.437	4.917	100
Rhamnetin 3-O-gentiobioside	318.282	6.041	3	6.45	0.928	76.564	-1.789	-4.439	-0.28	66.098
7- Methyl Physcion	298.295	4.762	0	4.25	2.31	349.201	-1.014	-3.604	0.021	85.985
Betulinic acid	456.707	2.921	2	3.7	6.162	276.235	-0.523	-3.003	1.34	93.764
4-dodecyl-1,3,8-trihydroxyanthraquinone	424.536	4.323	1	4.25	5.545	161.661	-2.466	-2.97	1.14	85.983
Vanillic acid	168.149	3.492	2	3.5	1.05	82.892	-0.85	-3.726	-0.748	67.43
Iscoscopoletin	192.171	5.145	1	4	0.869	665.921	-0.549	-3.015	-0.482	82.57
Gefitinib	446.908	4.144	1	7.7	4.123	629.347	0.073	-3.2	0.355	100
Wortmannin	428.438	4.71	0	11.2	0.294	168.243	-1.153	-4.458	-1.091	68.508
AZD5363	428.92	7.116	5	7.7	1.719	34.261	-1.141	-5.32	-0.186	64.483

Q P log Po/w (-2.0 to 6.5) Predicted octanol/water partition co-efficient log p; (range:-0.20 to 6.5)
Predicted IC50 values for blockage of HERG K^+ channels; (acceptable range: above-5.0)
QPP Caco-Predicted apparent Caco-2 cell permeability in nm/sec. Caco-2 cells is a model for the gut blood barrier; (nm/sec)\25-poor[500-great
Q P log BB-Predicted brain/blood partition coefficient (-3-1.2)
QPP MDCK-Predicted apparent MDCK cell permeability in nm/sec. MDCK cells are considered to be a good mimic for the blood–brain barrier; (nm/sec)\25-poor[500-great
Q P log KP-Predicted skin permeability; (-8.0 - -0.1)
Q P log KhsaPrediction of binding to human serum albumin; (acceptable range:-1.5 to 1.5)