# CHARACTERIZATION OF ANTIOXIDANT FRACTIONS IN CURRY LEAF (*Murraya koenigii* L.) AND MOLECULAR DOCKING OF SELECTED BIOACTIVE COMPOUNDS

By

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**(2017-11-001)**

**THESIS**

**Submitted in partial fulfilment of the requirement**
**for the degree of**

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**2019**

## DECLARATION

I hereby declare that the thesis entitled **"Characterization of antioxidant fractions in curry leaf (*Murraya koenigii* L.) and molecular docking of selected bioactive compounds"** is a bonafide record of research work done by me during the course of research and that it has not been previously formed the basis for the award to me of any degree, diploma, fellowship or other similar title, of any other University or Society.

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Certified that the thesis entitled **"Characterization of antioxidant fractions in curry leaf (*Murraya koenigii* L.) and molecular docking of selected bioactive compounds"** is a record of research work done independently by **Mr. Bhamare Deepak Prashant (2017-11-001)** under my guidance and supervision and that it has not previously formed the basis for the award of any degree, diploma, associateship, fellowship to him.

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# TABLE OF CONTENTS

# LIST OF TABLES

## LIST OF FIGURE

| | | |
|---|---|---|
| 15 | Hydrogen bond interaction of diabetes target fructose 1,6-bisphosphatase (2JJK) with with Valylmethionine | 50-51 |
| 16 | Hydrogen bond interaction of diabetes target human glucokinase (1V4S) with DL-2-aminooctanoic acid | 50-51 |
| 17 | Hydrogen bond interaction of diabetes target glycogen synthase kinase-3 (1Q5K) with Valylmethionine | 50-51 |
| 18 | Hydrogen bond interaction of diabetes target pyruvate dehydrogenase kinase (4MP2) with Flucoxetine | 51-52 |
| 19 | Hydrogen bond interaction of diabetes target human aldose reductase (3G5E) with Alpha-aminodiphenylacetic acid | 51-52 |
| 20 | Hydrogen bond interaction of diabetes target human multidrug resistance protein (2CBZ) with Histidinol | 51-52 |
| 21 | Hydrogen bond interaction of diabetes target dipeptidyl peptidase IV (1X70) with Histidinol | 52-53 |
| 22 | Hydrogen bond interaction of arthritis target nitric oxide synthase (4NOS) with Histidinol | 52-53 |
| 23 | Hydrogen bond interaction of arthritis target cyclooxygenase (4COX) with Histidinol | 53-54 |
| 24 | Hydrogen bond interaction of arthritis target vascular endothelial growth factor (3HNG) with Valylmethionine | 53-54 |
| 25 | Hydrogen bond interaction of arthritis target glucocorticoid receptor (1M2Z) with Histidinol | 54-55 |
| 26 | Hydrogen bond interaction of Alzheimer's target human beta secratse 1 (2XFJ) with histidinol | 54-55 |
| 27 | Hydrogen bond interaction of Alzheimer's target tau protein (1J1B) with Alpha-aminodiphenylacetic acid | 54-55 |
| 28 | Hydrogen bond interaction of Alzheimer's target human acetylcholinesterase with (4BDT) Valylmethionine | 55-56 |
| 29 | Hydrogen bond interaction of Alzheimer's target human butyrylcholinesterase (4BDS) with Valylmethionine | 55-56 |

# Abbreviations

| | |
|---|---|
| % | : Per cent |
| > | : Greater than |
| µg | : Microgram |
| β | : Beta |
| µl | : Microlitre |
| AchE | : Acetylcholinesterase |
| ADMET | : Absorption Distribution Metabolism Elimination Toxicity |
| AR | : Aldose redutase |
| BACE | : Beta-Secretase I |
| BchE | : Butyrylcholinesterase |
| $C^0$ | : Degree celcius |
| CHARMM | : Chemistry at Harvard Macromolecular Mechanics |
| COX | : Cyclooxygenase |
| CPBMB | : Centre for Plant Biotechnology and Molecular Biology |
| CYP2D6 | : Cytochrome P450 2D6 enzyme |
| DPP4 | : Dipeptdylpeptidase-4 |
| EPAC | : Exchange protein directly activated by CAMP |
| FbPase | : Fructose 1,6-bisphosphatase |
| GSK | : Glycogen Synthase Kinase |
| HMRP | : Human multidrug resistance protein |
| KAU | : Kerala Agricultural University |
| NOS | : Nitric acid synthase |
| PDK | : Pyruvate dehydrogenase kinase |
| PI3K | : Phosphoinositide 3- phasphate |
| PLK1 | : Polo-like kinase 1 |

# *Introduction*

# 1. Introduction

Curry leaf (*Murraya koenigii* L.) belonging to the family Rutaceae has been known for its immense medicinal properties like antioxidant, antidiabetic, anticancerous, analgesic, anti-inflammatory and hepatoprotective activities (Jain *et al.*, 2012). The therapeutic potential of curry leaf arises from its chemical constituents. Carbazole alkaloids are the most active compounds in curry leaf responsible for the antioxidant properties. Till date, 30 carbazole alkaloids have been reported in curry leaf (Nalli *et al.*, 2016; Chauhan *et al.*, 2017). Apart from carbazole alkaloids, phenols, flavonols, tannins, terpenes are present in curry leaf.

Curry leaf is one of the important plants used in traditional medicine. Curry leaf powder, oil and leaves are widely used in traditional medicine. It is used for the treatment of dysentery, stomach pain, diabetes, piles, reduce body heat, thirst, inflammation, and itching. External application of curry leaf on bruises, and eruption are beneficial and it is also used to treat bites of poisonous animals. It served as a potential anthelmintic and analgesic agent (Bhandari, 2012).

Curry leaf is well known for its nutraceutical properties. Antioxidant potential of curry leaf is explained by several researchers (Ramsevak *et al.*, 1990; Tachibana *et al.*, 2002; Rao *et al.*, 2007; Nigappa *et al.*, 2008; Aju *et al.*, 2017). Curry leaf has the potential to control diabetic conditions. (Yadav *et al.*, 2002) and anticancerous property of curry leaf was studied by Khanum *et al.* (2002). Potential of curry leaf in inhibition of neurodegenerative diseases is well recognised (Kumar *et al.*, 2010). Curry leaf is used as a remedy against inflammation (Darvekar *et al.*, 2011).

Curry leaf is widely used in food industry for improving the shelf life of processed food products. Das *et al.* (2011) used curry leaf extract as an antioxidant to prevent lipid peroxidation in goat meat. Adrika *et al.* (2015) evaluated the effect of curry leaf powder on packaging and quality of banana chips and observed superior physical and chemical qualities of stored chips, where curry leaf powder was used.

Oxidative stress is involved in several pathological conditions like cancer, diabetes, neurodegenerative diseases, and cardiovascular diseases. Oxidative stress occurs due to an imbalance between the production of reactive oxygen species and antioxidant defence system (Liguori *et al.*, 2018). Reactive oxygen species (ROS) are the reactive chemical species comprising a large group of oxygen-derived molecules produced by several endogenous and exogenous processes. The ROS generated due to

various metabolic processes can be scavenged by use of antioxidants. Synthetic antioxidant like Butylated hydroxyanisole is being used against ROS for a long time and it exhibited side effects (Hocman, 1988). Natural antioxidants also have the potential to cleave reactive oxygen species (Xu *et al.*, 2017) and natural antioxidants received much specific attention now-a-days as they exhibit no side effects when compared to synthetic antioxidants.

Though pharmacological and nutraceutical potential of curry leaf are well known, there is limited information available on the role of different bioactive phytocompounds of curry leaf behind curing diseases by interacting with target proteins of diseases. The identification of bioactive compounds from curry leaf will be helpful in finding out potential natural compounds against different diseases. Techniques like chromatography are widely used for the separation and identification of compounds in plants (Ingale *et al.*, 2017). Bioinformatics tools like molecular docking are now widely used for finding potential drug candidates from plants (Yi *et al.*, 2018).

Considering the importance of natural antioxidants and identification of bioactive compounds, the present study was carried out with the objective to characterize antioxidant fractions from curry leaf by *in vitro* assay and identification of most potent bioactive compound against various diseases, by LC/MS-MS and molecular docking analyses.

# Review of Literature

# 2. Review of Literature

Curry leaf (*Murraya Koenigii* L.) a popular spice crop grown commercially in south Indian states is known for its medicinal properties. This aromatic leafy spice is part of most of the cuisines prepared in southern India. The leaves are used to flavor a range of dishes and typically these are fried in oil until crisp to impart flavor to all types of curry preparations. Curry leaf is known as a miracle medicinal plant due to its therapeutic potential. Leaves, barks, and roots of curry leaf used for curing various ailments. Green leaves are used to treat piles, inflammation, inching, fresh cuts, dysentery, vomiting, burse, and dropsy. For curing general body aches roots are used, and the barks used in case of snakebite (Choudhury and Garg, 2007).

Curry leaf is known to possess several therapeutic compounds which impart different medicinal properties. Antioxidant compounds in curry leaf protect the cell from reactive oxygen species which may lead to the occurrence of diseases such as cancer, heart attack, and diabetes. This study aimed to find out the bioactive compounds with high antioxidant activity and to dock such bioactive compounds against the key proteins involved in diseases such as cancer, diabetes, Alzhimer, and arthritis. The relevant literature regarding various aspects of this study is reviewed in this chapter.

**Botany:**

## 2.1 ORIGIN AND DISTRIBUTION

Curry leaf (*Murraya koenigii* L.) is a native of India, Sri Lanka, and other South Asian countries. It is distributed throughout India and abundantly found from Sikkim to Garhwal, Bengal, Assam, Western Ghats and Kerala (Singh *et al.*, 2014). Apart from India, it is found in the Asian region like in moist forests of 500- 1600 m height in Guangdong, Bhutan, Laos, Nepal, Pakistan, Sri Lanka, Thailand, and Vietnamurraya. Curry leaf reached in Malaysia, South Africa, and Reunion Island through South Indian immigrants. They are rarely found outside the Indian sphere (Reddy *et al.*, 2018).

## 2.2 PLANT DESCRIPTION

Curry leaf is a small spreading, more or less deciduous shrub or small tree grows up to 6 m in height. The trunk of plants is short ranging from 15 to 40 cms in diameter, with smooth, greyish or brown bark and has a dense shady crown (Khosala and Prasad, 1974). The main stem is dark green to brownish with numerous dots on it.

The leaves are exstipulate, bipinnately compound, 15-30 cm long, rachis bears 11-25 leaflets in an alternate pattern, ovate lanceolate 4.9 cm long, 1.8 cm broad, with 2-3 cm-long petiole with an oblique base. The inflorescence is terminal cyme; each bearing 60-90 flowers. Fruits are small ovoid or subglobose, glandular, with thin pericarp enclosing one or two seeds which is spinach green (Gupta and Prakash, 2009). The seeds of the curry leaf are toxic in nature and not recommended for consumption (Gupta and Prakash, 2009; Gaholat *et al.*, 2014).

## 2.3 CHEMISTRY OF CURRY LEAF

Curry leaf contains different compounds such as carbazole alkaloids, flavanols, terpenes, lipids, etc. composition of these compounds greatly depends on part of curry leaf tree.

Extract of curry leaf contains moisture (66.3%), protein (1%), fat (1%), carbohydrate (16%), fiber (6.4%) and mineral matter (4.2%). Calcium, phosphorus and iron are predominant minerals found in curry leaf. Leaves contain vitamins such as nicotinic acid, vitamin C as well as carotene (Kumar *et al.*, 1999). Presence of oxalate reduces bioavailability of calcium and curry leaf contains total oxalate 1.352 per cent and soluble oxalate 1.155 per cent (Ananthasdamy *et al.*, 1960 and Walde *et al.*, 2005). Volatile oils and carbazole alkaloids are major constituents in curry leaf extract.

### 2.3.1 Phytocompounds in curry leaf

Curry leaf is a rich source of several phytocompounds such as phenols, flavanols, flavonoids, alkaloids, tannins, saponins. Hema *et al.* (2011) done determination of bioactive compounds from ethanolic extract of *Murraya koenigii* using Gas chromatography and mass spectrometry (GC-MS). They identified 13 compounds viz. Propane, 1,1,3-triethoxy-;1,2-Ethanediol, monoacetate; 1-Methyl-pyrrolidine-2-carboxylic acid; Ethyl a-d-glucopyranoside; Pentadecanoic acid, 14-methyl-, methyl ester; n-Hexadecanoic acid; Hexadecanoic acid, ethyl ester; Oleic acid, methyl ester; Phytol; 9,12-Octadecadienoic acid (Z, Z); ç-Himachalene; 1,2-Benzenedicarboxylic acid diisooctyl ester and Isolongifolene, 4,5-dehydro.

Singh *et al.* (2011) characterized flavanols in curry leaf using LC-MS-MS analysis. Freeze dried curry leaf powder was extracted using acetone, methanol, ethanol. Flavanol content of each sample was estimated using HPLC. Predominant flavanols identified were myricetin-3-galactoside, quercetin-O-pentohexoside,

quercetin diglucoside, quercetin-3-O-rutinoside, quercetin-3-glucoside, quercetin-3 acetylhexoside, quercetin- O-xylo-pentoside, kaempferol-O-glucoside, and kaempferol aglucoside. The yield was higher for extraction with ethanol while acetone extract exhibited higher antioxidant activities.

Vinod *et al.* (2013) studied the chemical composition of aqueous extract of curry (AEC) leaf by Gas chromatography-Mass spectrometry (GC-MS) method. They identified organic compound, aromatic hydrocarbons and monoamine alkaloids from AEC viz. Benzene; 1-ethyl-3-methyl; Tropylium ($C_9H_{11}$); Ethyl, 2-phenyl; Styrene; Tropylium ($C_7H_7$); 6-methylenecyclohexa- 1,2,4-triene ($C_7H_6$); Tropylium ($C_7H_5$), Tropylium ($C_7H_4^{2+}$); 2,4-Cyclopentadienide and Tropylium ($C_5H_5^+$).

Ghasemzadeh *et al.* (2014 a,b) assessed total flavanoids and phenol content in curry leaf from three different locations using reverse phase HPLC. Total phenol and flavanoids content were highest in Kelatin (14.371 mg/g DW and 3.771 mg/g DW) followed by Selangor (12.272 mg/g DW and 3.146 mg/g DW) and Johor (12.02 mg/g DW and 2.801 mg/g DW). Content of catechin (0.325 mg/g DW), quercetin (0.350 mg/g DW), naringin (0.203 mg/g DW), epicatechin (0.678 mg/g DW) and myricetin (0.703 mg/g DW) was found higher in Kelatin. Later, they optimized the protocol for extraction of flavonoid compounds with pharmaceutical quality using response surface methodology.

Balasubramanian *et al.* (2014) carried out GC-MS analysis of curry leaves. Methanolic extract of curry leaf was subjected to GC-MS. GC-MS chromatogram showed the presence of five compounds viz. Alpha-caryophyllene; Benzene, 1-dimethylamino-4- (2-cyano-2-phenylethenyl, 2-phenyl-4-quinolinecarboxamide; phenanthrene 9,10-diethyl-3,6-dimethoxy; 10h-phenoxaphosphine, 2- chloro-8-ethyl-10-hydroxy; and 1,5-diformyl-2,6-dimethoxyanthracene at retention time 22.15, 45.88, 45.52, 45.52, 45.52 min respectively.

Igara *et al.* (2016) carried out phytochemical and nutritional profiling of curry leaf. They had estimated concentration of phytochemicals like flavanoids (7.43 mg/100g), phenols (4.25 mg/100g), alkaloids (1.90 mg/100g), tannin (0.86mg/100g) and saponin (2.50 mg/100g). The concentration of different vitamins were Vitamin A (6.04 mg/100g), Vitamin C (0.04 mg/100g), thiamine (0.89 mg/100g), riboflavin (0.9mg/100g), niacin (2.73 mg/100g) and Vitamin E (0.03 mg/100g). Salomi and Manimekalai (2016) studied the effect of different solvents on concentration of phytocompounds in curry leaf. Alkaloid concentration was more in ethanolic extract

(49.09 mg/g). The aqueous extract was rich in concentration of tannins (114.76 mg/g). The concentration of total phenol was high in acetone extract (459.63 mg/g) while least in aqueous extract (139.26 mg/g). Saponin concentration was high in hexane extract (70.68 mg/g).

#### *2.3.1.1 Carbazole alkaloids*

Carbazole alkaloids are any alkaloids that seat on carbon skeleton which is neutral or phenolic originated from anthranilic acid. The first carbazole was identified in 1972 by grabe and glazer from Coal Tar. Study of carbazole chemistry accelerated in 1920 as was used in European dyestuff industries. Carbazole as plant products were unknown until discovery of two pyridocarbazoles ulein and olivicin (Ondeti and Deulofeu, 1961). Murrayanine was the first member of carbazole alkaloid that was distinct from pyridocarbazoles. Curry leaf is known to be the richest source of phytocarbazoles so far reported. Chakarborty *et al.* (1964) discovered first carbazole in curry leaf from stem i.e. girinimbine. Later Murrayanine was isolated from stem of curry leaf plant by Chakarborty *et al.* (1965).

Discovery of carbazole alkaloids from curry leaf progressed at a high rate in the 1970s. Joshi *et al.* (1970) elucidated the structure of two previously reported carbazole i.e. girinimbine and murrayanine and two new carbazole alkaloids i.e. isomahanimbine and koenimbidine using thin layer chromatography and NMR. Anwer *et al.* (1972) synthesized DL-O-Methylmahanine, 8-hydroxymahanimbine, and pyranylcarbazole from carbazole. Mahanimbine was isolated from roots of curry leaf by Roy and Chakraborty (1974). The structure of new carbazole alkaloids isomurryazoline was identified from stem bark (Bhattacharya *et al.*, 1982). Mukherjee *et al.* (1982) identified another carbazole alkaloid, mukonicine from an ethanolic extract of curry leaf using column chromatography using alumina. The purity of compound was confirmed by TLC using various solvent systems. Bhattacharya and Chakarborty (1982) revealed structure of Mukonal. It is a biogenetic intermediate of pyranocarbazole alkaloids. Based on chemical and physical transformation using UV, IR, H-NMR, and C-NMR structure was found as 2-hydroxy-3-formyl carbazole.

Fiebig *et al.* (1983) identified cytotoxic carbazole alkaloid form curry leaf. Koeniline showed cytotoxic activity against some cell cultures and its structure was studied by spectroscopy and partial synthesis of compound was carried out from murrayamine. Koeniline showed major cytotoxicity against KB cell culture test

systems. Later, Adesina *et al.* (1988) reported new bioactive compounds 3-methyl carbazole and glycozoline respectively in curry leaf.

Ito *et al.* (1993) analysed alkaloid constituents in root and stem bark of curry leaf. They subjected acetone extracts of root and stem bark for fractionation using silica gel column chromatography followed by preparative TLC. Structures of the isolated compound was discovered by using HR-MS. They could find three new monomeric carbazole alkaloids and five novel binary carbazole alkaloids named mukoenine-A (1), -B (2), and -C (4), and murrastifoline-F (8), bis-2-hydroxy-3- methylcarbazole (9), bismahanine (11), bikoeniquinone-A (12), and bismurrayaquinone-A (13), respectively. Apart from these, they could find 16 reported carbazoles and carbazolequinones from the extract.

Ramsevak *et al.* (1999) reported three new carbazole alkaloids from *Murraya koenigii* L. namely mahanimbine, murrayanol and mahanine. Acetone extract of leaves was subjected to fractionation using vacuum liquid chromatography (VLC). Resulting fractions were analysed for presence of bioactivity. Fraction C and E were subjected to Medium-pressure liquid chromatography (MPLC) on silica gel. Fraction C yielded five subtractions of which IV was bioactive and it was further purified by preparative TLC while fraction E yielded five fractions of which III and IV were bioactive and purified by preparative TLC. Finally, the three compounds were analysed by $^{1}H$ and $^{13}C$ NMR spectra and found spectroscopically pure viz. mahanimbine, murryanol and mahanine.

Nutan *et al.* (1999) identified a new carbazole alkaloid, bismurrayafoline E. from curry leaf. Alcoholic extract of curry leaf was subjected to four-step modified (liquid-liquid) Kupchan partioning process. Further, vacuum liquid chromatography was performed followed by purification with silica gel TLC yielded compound 1 (2 mg). Later compound was subjected to 1H and $^{13}C$ NMR spectra and the compound was identified as bismurrayafoline -E.

Wang *et al.* (2003) isolated two new carbazole alkaloids from the aerial part of *Murraya koenigii*. The air dried plant material was extracted using 95 per cent ethanol and extract further partitioned with $H_2O$ and chloroform. A portion of this extract was subjected to silica gel column chromatography, further eluted with petroleum ether-EtOAc in ratio of 9:1 to 4:6 (v/v) to allow 10 fractions. Later, Fraction 6 (10.2 g) was subjected to repeated silica gel column chromatography elution with and Sephadex LH-20 elution with methanol to get koenigine (560 mg) and compound 2 (52 mg). Fraction 8 (1.2 g) was further purified using silica gel column chromatography eluted with

$CHCl_3$-Me2CO (85:15, v/v) and with Sephadex LH-20 column eluted with Methanol to yield compound 1 (7.5 mg). Compounds were detected using IR spectra and mass spectrometry followed by 1D and 2D NMR. Novel compound murrayanine along with 8,8-biskoenigine were identified.

Tachibana *et al.* (2003) isolated carbazole alkaloids isolated from leaves of *Murraya koenigii.* Air-dried leaves of *Murraya koenigii* was extracted using dichloromethane and 70 per cent acetone and evaporated. The dried extract was partitioned using ethanol to yield soluble (53.6 g) and water soluble (53.6 g) fractions. The dichloromethane fraction was fractioned using silica gel column chromatography and eluted using a mixture of $CH_2Cl_2$ and acetone which yielded 11 fractions. These fractions were further separated by using preparative HPLC with reverse phase C-18 Column. Compounds individually purified using column chromatography and Sepadex LH-20 column yielded different compound and detection was done using $^{1}H$ and $^{13}C$ NMR and mass spectrometry. Compounds identified were koenimbine, O-methylmurrayamin A, O-methylmahanine, isomahanine, bismahane, bispyrayafoline, and a new dimeric carbazole 8,10c-[3,3c,11,11c-tetrahydro-9,9c-dihydroxy-3,3c,5,8c-tetramethyl-3,3c-bis(4-methyl-3pentenyl) bipyrano [3,2-a]carbazole.

Rao *et al.* (2005) identified two carbazole alkaloids possessing high antioxidant activity. Oleoresin was extracted from dehydrated curry leaf powder and subjected to silica gel column chromatography. The material was eluted using hexane and subsequently polarity of solvent was increased using ethyl acetate. Similar fractions were collected and subjected to sub-fractionation yielded five compounds. Compounds were identified using $^{1}H$ and $^{13}C$ NMR spectra. These compounds were mahanimbine and koenigine.

Ma *et al.* (2013) identified four new carbazole alkaloids from curry leaf. The whole plant of curry leaf was crushed with 95 per cent ethanol and the extract was dehydrated. The extract was subjected to several rounds of column chromatography, thin layer chromatography (TLC) and preparative HPLC for separation, fractionation and purifications of compounds. Total 18 compounds were identified out of which 14 were reported earlier and four were novel. The novel compounds were N-benzyl carbazole-A, N-benzyl carbazole-B, iso-koenidine.and iso-koenigine. Later, Tan *et al.* (2015) isolated six new carbazole alkaloids. Air dried bark and leaves were powdered and extracted using n-hexane and dichloromethane. Further compounds were isolated through column chromatography and mixture of compounds were separated by

preparative TLC. Finally compounds were analysed using 1D and 2D NMR. Six new compounds viz. murrastanine-A, murrastinine-A, -B, -C, murrayatanine-A and bismahanimboline and 24 reported compounds were obtained. Nali *et al.* (2016) found four new carbazole alkaloids from stem and leaves of curry leaf. Various chromatographic and spectrometric techniques were used for isolation and yielded total 24 compounds. Analysis of 1D and 2D were done for samples and Murrayakonine A-D was identified.

### *2.3.2 Phytocompounds in volatile oil*

Curry leaf is a good source of volatile oil. Variation in composition and recovery of volatile oil is due to dehydration (Madalgeri *et al.*, 1996). Terpenes are the predominant compounds found in essential volatile oil of curry leaf which is used as a flavouring agent (MacLoed and Pieris, 1982). Gas chromatography and mass spectrometry revealed presence of more than 60 monoterpene and sesquiterpenes in six different species of curry leaf. Wong and Tie (1993) studied the chemical composition of curry leaf and identified 63 compounds. Main constituents were β-phelleandrene (24.4%), α-pinene (17.5%), β-caryophellene (7.3%) and terpene-4-ol (6.1%). Mallavarapu *et al.* (2000) found out 48 essential compounds of leaf oil, 42 compounds of fruit oil accounting for 98.5%. The major constituents of leaf essential oil were β-phelleandrene (50.1%), α-pinene (9%), (E)- β-ocimene (7.1%, ), α -phelleandrene (6.1%) and β-caryophellene (4.9%). In another study, Raina *et al.* (2002) found the presence of 34 compounds in leaves of which 97.4 per cent compounds was from oil and major components were α-humelene (1.2%), gamma-terpenene (1.2%), terpenen-4-ol (1.3%), limonene (5.4%), β-caryophellene (5.5%), β-pinene (9.8%), sabinene (10.5%) and α-pinene (51.7%).

The difference in agroclimatic conditions directly influence chemical composition of curry leaf leading to variation. Curry leaf grown in Nigeria was found to have 89.1 per cent oil and main compounds were β-caryophyllene (20.5%), bicyclogermacrene (9.9%), α-cadinol (7.3%), caryophyllene epoxide (6.4%), β-selinene (6.2%) and α-humulene (5%) while curry leaf grown in Sri Lanka possessed 53 compounds and major compounds were β-thujene (5.8%), β-phellandrene (18.9%), (E)-β-ocimene (12.7%), β-caryophyllene (23.3%), α-humulene (4.3%) and β-bisabolene (3.14%) (Onayade and Adebajo, 2000; Paranagama *et al.*, 2002).

## 2.4 PHARMACOLOGICAL PROPERTIES OF CURRY LEAF

Curry leaf is considered as an important plant in Ayurveda due to the presence of a variety of pharmacological properties like anticancer, hyperglycaemic, anti-inflammatory, Alzheimer's disease therapy, cardioprotective, antiobesity and antihyperlipidemic, antipyretic, antiulcer, antidiarrheal, anthelmintic, hepatoprotevtive, antimicrobial, antifungal and immunolomudulatory activity (Vandana *et al.*, 2012; Handral *et al.*, 2012).

### 2.4.1 Antioxidant and free radical scavenging property

Curry leaf is a rich source of the antioxidant compounds and several researchers have reported antioxidant potential of curry leaf. Ramsevak *et al.* (1999) reported antioxidant activity of carbazole alkaloids. Three antioxidants were isolated from acetone extract of curry leaf using bioassay guided fractionation mahanimbine (I), murrayanol (II), and mahanine (III). Highest antioxidant activity was found in mahanimbine (33.1µg/ml) while mahanine possessed highest antiinflammatory activity.

Tachibana *et al.* (2002) evaluated the antioxidant potential of 12 carbazole alkaloids from curry leaf using oil stability index and radical scavenging assay using DPPH system. Oil stability index was found high for mahanine (10.4) followed by Euchrestine B (9.01), isomahanine (8.81), bismahanine, bispyrayafoline and 8, 10'-{3,3', 11, 11'-terahydro-9, 9' dihydroxy- 3,3',5, 8'-tetra methyl –3,3'-bis (4-methyl-3-pentenyl)} bis pyrano (3,2 a) and it was significantly greater than that of Butylated Hydoxytoluene. Radical scavenging activity was higher for mahanine than the α-tocopherol. Again five carbazole alkaloids were isolated from methylene chloride extract of curry leaf. These were Euchrestine B(1), bismurrayafoline E(2), mahanine (3), mahanimbicine (4) and mahanimbine (5). The order for oil stability index was 1, 3 > tocopherol > BHT > 2 > 4, 5 while for DPPH inhibition order was ascorbic acid > 2 > 1,3 and tocopherol > BHT > 4,5.

Roa *et al.* (2007) identified two carbazole alkaloids with high antioxidant activity from the oleoresin of *Murraya koenigii.* Oleoresin extracted by using acetone showed higher inhibition of DPPH (83.4%). The DPPH inhibition for methanol extract of curry leaf was 18.0 per cent , while for aqueous extract of curry leaf, DPPH inhibition was 14.3 per cent. volatile oils recorded less inhibition of DPPH with 13.6 percent. Among compounds isolated from curry leaf, koenigine showed maximum inhibition of DPPH with 87.1 per cent.

Nigappa *et al.* (2008) isolated antioxidant peptide of 35 kDa from curry leaf. Protein was extracted from curry leaf powder using Tris-buffer and ammonium sulphate (65 %) and gel filtration on Sephadex G-75 column was carried out. It gave three peaks PI, PII and PIII which further tested for lipid peroxidation assay. The PII found to be the best for inhibition of lipid peroxidation and further rechromatographed on Tris-buffer and ammonium sulphate (65 %) and gel filtration was done on Sephadex and reverse phase HPLC. The molecular weight of the antioxidant protein of curry leaf (APC) PII was ~35 kDa. The APC at 0.8 µm showed inhibition of lipid peroxidation by 71 per cent. The APC inhibited DPPH and hydroxyl ion at concentration of 150 fold lesser than the concentration at which BHA and α-tocopherol inhibited DPPH and hydroxyl ion (4 µM). Nigappa *et al.* (2008) studied antioxidant properties of different extracts (Alcohol, water, alcohol:water, chloroform extract, and hexane) of curry leaf using various assays. Antioxidant activity and free radical scavenging potential was highest for alcohol:water (1:1) extract. It inhibited lipid peroxidation of membrane by 71 per cent, scavenged 90 per cent hydroxyl ions and inhibited DPPH with 92 per cent inhibition.

Gupta *et al.* (2009) analysed antioxidant potential of green leafy vegetables (GLV) in India. Methanolic extracts *of Amaranthus* sp., *Centella asiatica*, *Murraya koenigii* and *Trigonella foenum graecum* were studied for antioxidant potential at different concentrations using three methods *viz.* phosphomolybdenum method, free radical scavenging activity by 1,1-diphenly-2-picryl hydrazyl (DPPH), reducing power and ferrous ion chelating activity. *Murraya koenigii* possessed the highest antioxidant activity (2,691.78 µmol of ascorbic acid/g sample) while *Centella asiatica* recorded 623.78 of ascorbic acid/g sample. The DPPH scavenging activity was also high in *Murraya koenigii*.

Aju *et al.* (2017) evaluated the antioxidant activity of *Murraya koenigii* (L.) Spreng using different *in vitro* methods. Dehydrated curry leaf powder was extracted using methanol (63° C), hydro alcohol (80° C) and water (100° C) in soxhlet apparatus and later subjected to total antioxidant assays (DPPH radical scavenging assay, reducing power assay, nitric oxide radical scavenging activity, hydrogen peroxide scavenging activity assay, hydroxyl radical scavenging assay, superoxide radical scavenging activity assay). It was found that hydro-alcohol extract possessed highest total antioxidant activity (22.94±0.01 µg of ascorbic acid per mg of extract) than water (17.28±0.31 µg of ascorbic acid per mg of extract) and methanol (8.61±0.01 µg of

ascorbic acid per mg of extract).

### 2.4.2 Anticancer property

Khanum *et al.* (2000) studied the anticarcinogenic effects of curry leaf in dimethylhydrazine-treated rats. Rats were divided into four groups based on diet. Rats in first group were the untreated control which were fed with casein diet, second group rats were fed with diet treated with dimethyl hydrazine hydrochloride (DMH) (carcinogenic compound), third group rats with diet substituted with curry leaf and the fourth group with leaf-substituted di*et al*ong with DMH. All rats were kept in steel cages and after 12 weeks they were sacrificed and liver, kidney femur excised for biochemical analysis. It was observed that vitamin A level in liver increased (Highest in group two rats (21.39±1.83 µg retinol equivalent/g liver), least in group three (17.43±1.87 µg retinol equivalent/g liver)) while there was increase in gamma-glutamyl transpeptidase (index for precancerous changes in tissues) in group three (6.20±0.18) and least in group one (0.99±0.09). Similarly, micronuclei formation was highest in group three (2.16±0.23%) and least in group one (0.46±0.08a). It indicated high anticancerous potential of curry leaf to reduce carcinogen.

Mahanine, a carbazole alkaloid present in *Murraya koenigii* is reported to induce apoptosis in myeloid cancer cell line HL-60 at low concentration of 10 µM. Mahanine caused DNA fragmentation, changes in nuclear morphology, activation of caspase activity and release of cytochrome C into cytsol. In a Flow cytometry study it was observed that mahanine reduced mitochondrial membrane potential and caused disturbance in cell cycle progression which led to apoptosis. Mahanine reduced cell survival *via* caspase-3 activity (Roy *et al.*, 2004).

Kok *et al.* (2012) studied the antitumor promoting and antioxidant activity of carbazole alkaloid Girinimbine isolated from curry leaf. The antitumor activity was determined by counting inhibition of early antigen (EA) of Epistine Beta Virus (EBV) on Raji cell surface. Raji cells are B-human lymphoblastoids cells. Girinimbine powerfully inhibited induction of EA of EBV almost more than 90 per cent at concentration of 16 and 32 µg/mL but at low concentration the inhibition rates were moderate. Girinimbine showed high antioxidant activity as well as high rate of superoxide inhibition.

Paterson and Verghese (2015) showed antitumor and toxicity effects of curry leaf extracts. The dehydrated curry leaf powder was extracted using methanol and

extract was dissolved in methanol again to study (Ferric reducing antioxidant power and Free radical scavenging activity). Aqueous curry leaf extracts (CLE) were added to cell growth medium of Caco-2 colon adenocarcinoma cell line and HepG2 human liver cell line and incubated at 12 hr and 24 hrs. The activity of CLE against cell lines were determined using different analysis (Release of lactate dehydrogenase, Glutathione-s-transferase activity, catalase activity, and superoxide dismutase). Aqueous curry leaf extracts inhibited DPPH at $IC_{50}$ of 83.85 mg/ml while ferric iron reducing antioxidant potential was 0.81 µmol $Fe^{+2}$/g. Release of LDH was higher (55%) in Caco2 cells treated with 0.8 µg/mL of CLE. This infers the high cytotoxic potential of CLE. Maximum release of LDH was 15.74 per cent in HepG2 cells treated 0.8 µg/mL of CLE. This infers low toxicity of CLE. Enzyme activities (Glutathione-s-transferase activity, catalase activity, and superoxide dismutase) of Caco2 cells were higher for 12 hrs incubated lines than 24 hrs incubated which infers the high cytotoxic potential of CLE with less incubation period. Histone related DNA fragmentation was higher in Caco2 lines (12 hrs) while in case of HepG2 it was higher for 24 hrs which infers that treatment of CLE for 12 hrs is optimal with less toxicity. This study suggested that curry leaf has potential to cure colon cancer with slight toxicity to liver.

Ismail *et al.* (2016) demonstrated cytotoxicity and proteasome inhibition of breast cancer cells by alkaloid extract of *Murraya koenigii* leaves. The total alkaloid extract (TAE) of curry leaf was prepared by extracting dried curry leaf powder with methanol in the Soxhlet apparatus. The breast cancer cell line MDA-MB-231 was treated with TAE at different concentrations of 10, 20, 30, and 40 µg/mL After 24 hrs, the cells were harvested and stained with Annexin V-FITC and PI. Later cells were observed in inverted fluorescence microscope and inhibition of 20S proteosome was assessed. It was found that Trypsin-like proteolytic activity of proteasome was inhibited by TAE at $IC_{50}$ 162 µg/mL and cell viability of breast cancer cells was decreased by TAE at $IC_{50}$ of 14.4 µg/mL and cell cycle was arrested at "S" phase by TAE (32 µg/mL) which led lead to apoptosis.

### 2.4.3 Hyperglycaemic property

Yadav *et al.* (2002) demonstrated hypoglycemic and antihyperglycemic activity of *Murraya koenigii* leaves in diabetic rats. Diabetes was induced in rats using alloxan (154mg/dl mild diabetes) and Streptozotocin (35 mg/kg IP moderate diabetes). The rats were fed with diet having different dosage of curry leaf (5, 10 and 15%) and the blood

glucose level was monitored. The reduction in blood glucose level for normal rats were negligible while rats fed with diet containing different doses of curry leaf (5, 10 and 15%) showed 13.1, 16.3 and 21.4 per cent of blood glucose level reduction which showed hypoglycaemic effect.

Vinuthan *et al* (2004) studied the effect of extracts of *Murraya koenigii* leaves on blood glucose and plasma insulin level in alloxan-induced diabetic rats. The curry leaf extract (CLE) was prepared using water and methanol. The rats were divided into four groups (diabetic T0, diabetic T1, diabetic T2 and diabetic T3). Diabetic T0 was kept as control while diabetic T1 group was fed with an oral dose of aqueous extract of curry leaf (600 mg/kg) and diabetic T2 group was fed with methanol extract of curry leaf at 200 mg/kg of Rat (DMSO used for delivery of methanol extract) and T3 was DMSO control. After 0, 7, 14, 21, 28, 43 and 58 days, level of glucose and insulin were checked. The glucose and insulin concentration in T0 and T3 group was similar however in T1 and T2 there was a significant difference. Due to alloxen administration, glucose level was increased in blood. The blood glucose level was controlled by aqueous and methanol extract of curry leaf. It also showed significant increase in plasma insulin levels which helped in regulation of glucose. Similarly, Aruslevan *et al.* (2006) reported antidiabetic effect of ethanolic extract of curry leaf in Streptozotocin induced rats. The blood glucose level was significantly reduced in rats treated with curry leaf extract due to stimulation of insulin by extract while Lawal *et al.* (2008) demonstrated antidiabetic activity of aqueous extract of curry leaf in alloxen induced rats.

**2.4.4 Anti-inflammatory property**

Darvekar *et al.* (2011) proved anti-inflammatory potential of curry leaf. The ethanol extract, cholorofom extract, and petroleum ether extract were injected to Carrageenan induced mice. Commercial drug Ibuprofen recorded the highest inhibition with 60 per cent. Among curry leaf extracts, ethanol extract recorded highest inhibition of paw edema with 52 per cent followed by petroleum ether with 39 per cent and chloroform extract with 36 per cent. Mathur *et al.* (2011) also studied anti-inflammatory potential of curry leaf. The methanolic extract of curry leaf showed better inhibition of paw edema and 9, 12-octadecadienoic acid, a compound isolated from methonolic extract of curry leaf showed 85 per cent of paw edema inhibition than aspirin (68.62%), a standard anti-inflammatory drug at a dose of 150 µg/mL.

### 2.4.5 Alzheimer's disease therapy

Curry leaf has potential to cure Alzheimer's disease (Jain *et al.*, 2012). Kumar *et al.* (2010) demonstrated to potential of Mahanimbine (carbazole alkaloid from curry leaf) to inhibit acetylcholinesterase. The curry leaf powder was extracted using methanol. The methanol filtrate was further extracted using petroleum ether and choloform. The compound was isolated and purified by column chromatography and structure was confirmed using NMR. The acetylcholine inhibition was evaluated using bioactivity guided TLC and enzymatic assay. Several inhibitory spots were observed on TLC without any false positive spots. Enzymatic assay showed inhibition of acetylcholinestarase by methanol, petroleum ether, chloroform extract at $IC_{50}$ value of 0.15 ± 0.01 mg/mL, 0.07 ± 0.04 mg/mL, 0.12 ± 0.02 mg/mL and 0.03 ± 0.09 mg/mL, respectively. The petroleum ether extract showed the highest reducing ability.

Mani *et al.* (2012) studied protective effect of total alkaloid extract of *Murraya koenigii* leaves on dementia. Dementia is a condition of neurodegeneration where cognitive functions decline. Alzheimer's is one type of dementia. Total alkaloid extract was isolated from curry leaf (MKA) through methanol extraction. The Extract in different concentrations viz. 10, 20, 30 mg/kg was given to rats of each group for 15 days and retention memory was recorded on 16$^{th}$ day. Another group of rats was treated with scopolamine and diazepam to induce amnesia and on 16$^{th}$ day retention memory was recorded. Significant improvement in memory scores of young and aged mice was observed in rats treated with 20 and 30 mg/kg MKA as well as it reverted scopolamine and diazepam induced amnesia. The Cholinesterase activity in brain also reduced which is good sign in treatment of Alzheimer's.

## 2.5 REACTIVE OXYGEN SPECIES AND DISEASES.

### 2.5.1 Reactive oxygen species

Reactive oxygen species (ROS) are chemically reactive chemical species comprise of a large group of oxygen-derived small molecules. It includes nonradical and radicals species. Non-radical species are molecules of oxygen such as radicals such as superoxide singlet oxygen ($^1O_2$), hypochlorous acid (HOCl), hydrogen peroxide ($H_2O_2$) and ozone ($O_3$). Radicals are short lived, electrophilic and reactive molecules which have an unpaired electron in outermost shell and these are superoxide ($O_2$), peroxyl ($RO_2$) and hydroxyl (OH) (Jones, 2008). Formation of these molecules is multistep process. In initial step, molecular oxygen transferred one electron to form

superoxide anion which later used for hydrogen peroxide formation. Remaining steps include formation of peroxynitrite from reaction of superoxide with nitric oxide, formation of hypochlorous acid from hydrogen peroxide catalysed by peroxidase and generation of hydroxyl radical via iron catalysed Fenton reaction (Honeywell and Gutteridege, 2007).

The production of ROS at particular site is a result of balance between productions from enzymatic and non-enzymatic sources. The imbalance in this mechanism resulted in oxidative stress. The imbalance may be due to an inadequate antioxidant activity or may be due to overproduction of ROS. Reactive oxygen species ardently interact with a large variety of cellular molecules such as proteins, lipids, nucleic acid and small molecules and reaction associated with these cellular molecules can be altered due to imbalance in production of ROS leading to disease and aging (Liochev, 2013). Oxidative stress is involved in induction of many diseases which in turn is driven by ROS. Oxidative stress occurs due to overproduction ROS from different sources. Among all radicals, superoxide molecules ($SO_2$) are short lived, less stable and can not cross cell membranes while its dismuted product hydrogen peroxide can diffuse through biological membranes and cause damage to membranes.

2.5.2 Reactive oxygen species and cancer

Cancer is a complicated process includes a series of molecular and cellular events which results in transformation of normal cells into cancerous cells. These events include permanent stimulation to cell proliferation, resistance to cell death, loss of mitotic control, replicative immortality, metastasis and invasion activation, angiogenesis, metabolic deregulation and genetic instability (Hanahan and Weinberg, 2011).

Development of cancer is three step process viz. initiation, promotion and progression and ROS are involved in all three stages. Initiation of tumour formation occurs due to irreversible changes in DNA like point mutations and chromosomal aberrations. Long-time accumulation of these changes is important for tumour initiation as well as progression. Increase in level of DNA lesion has been associated with occurrence of various cancers (Sosa *et al.*, 2013). Exposure to various ionizing radiation and chemical agents (carcinogens) are reported to damage the genome and both are potential sources of ROS which mediate part of DNA damage. Reactive oxygen species promote formation of pyrimidine adducts as well as alkyl radical formation in thymine. It also causes breaks in DNA strands by reacting with sugar

moiety and finally all these effects cause mutation and rearrangement in chromosome (Ameziane-El-Hassani *et al.*, 2010; de Oliveira *et al.*, 2012). Reactive oxygen species modulates signaling pathways by interacting with surface and intracellular receptor which causes physiological disruption in various mechanism related to apoptosis, angiogenesis, and proliferation.

Cancer promotion is related to action of promoters. Promoters can be endogenous or exogenous. These are the molecules which can activate reversible mechanism of inflammation, impair cell death, stimulate cell multiplication and can cause gene alterations. Reactive oxygen species interfere with these processes and acts as initiator and promoter. Tumour formation occurs due to the loss of control on cell growth regulatory mechanism. Reactive oxygen species cause oxidative damage to enzyme that regulates activity of cell cycle. Also, ROS activates tyrosine phosphatase like phosphatase and tensin homolog (PTEN) and phosphotyrosine-binding domain (PTB-1) which causes hyperactivation phosphoinositide-3 phosphate and AKT pathways that are involved in cancer (Meng *et al.*, 2006; Gebremedhin *et al.*, 2013). The final step in cancer tumour progression is associated with malignant tumour formation which is a result of genomic instability, increased mortality, angiogenesis, and invasiveness. High proliferation of tumour is due to the release of pro-angiogenic factors which activate endothelial progenitors leading to the multiplication of vascular endothelial cells. Proteins like HIF-1 plays an important role in these processes and these and HIF is regulated by ROS (Gao *et al.*, 2002; Comito 2011).

Pathogenesis studies in breast cancer has revealed that DNA contains numerous base modifications. The increased level of 8-OHdG (a modified base) found in in early stage cancer cells in several cases of breast cancer. These modifications are due to the action of ROS and hence it is reported to play important part in early phases of carcinogenesis (Okoh *et al.*, 2011).

Colon cancer is the world third most diagnosed cancer in men and second frequently observed in women (Haggaer and Boushey, 2009).. Epithelial cells which line the bowel are the originator for colon cancer. Rapid division and high metabolic rates are the common characteristics of these cells and. Hydrogen peroxide damages cells from lower crypt section of bowel. Since the cells in colon are rapidly dividing they are more susceptible to DNA damage. Mutation occurs due to damaged DNA and these are carried forward to the next generation during division and cannot be repaired (Folesinski *et al.*, 2004; Oberreurner and Moscher, 2005).

The lack of oxygen supply to tissue (hypoxia) is a common characteristic of pancreatic cancer. The activation of pancreatic stellate cells (PSC) is the main reason for desmoplasia which is an important characteristic of pancreatic cancer (Hwang *et al.*, 2008). Let *et al.* (2014) showed that ROS play an important role in pancreatic stellate cell activation. Reactive oxygen species destabilizes HIF-α and upregulates Glil expression which leads to hypoxia and also activates PSC cells. These cells (PSC) produces soluble factors like IL-5, VEGF-A, and SBF-I which ultimately favours pancreatic cancer invasion. ROS is also involved in upregulation of AKT /mTOR pathway of pancreatic cancer cells. (Flonnie *et al.*, 2015).

Many evidences have suggested that malfunctioning of cellular metabolism is a major reason for prostate cancer occurrence and leads to formation of malignant cells. This impaired cellular metabolism is due to instability in nuclear and mitochondrial genome. The instability in genome occurs due to mutation in DNA by ROS (Valko *et al.*, 2004). The mutation in tumour suppressor genes also leads to alteration in cellular processes which results in aberrant growth of cell and formation of prostate cancer (Lee and Muller, 2010).

#### *2.5.2.1 Molecular targets identified for cancer*

#### *A. 17β hydroxysteroid dehydrogenase*

17β-hydroxysteroid dehydrogenase (17-beta HSD) is a group of enzymes that belongs to alcohol oxidoreductase involved in steroid metabolism. It catalyses the interconversion of dehydroepiadrosterone (DHEA) and androstenediol, androstenedione and testosterone, and estrone and estradiol. Estrogen is produced from adrenal androgens in order to activate breast cancer cells. This reaction includes several enzymes such as steroid sulfatase, aromatase, 17-beta HSD. In estradiol biosynthesis 17-beta HSD catalyses last step. The level of 17-beta HSD is found to be significantly high in patients of breast cancer where it supplies estrogen for growth and development of cancerous cells (Hilbon *et al.*, 2017).

#### *B. Polo like kinase-1*

Polo like kinase-1 (Plk) is a threonine/serine protein kinase which found in eukaryotic cells. Humans Plk family consist of five members: PLK1, PLK2, PLK3, PLK4, and PLK5. Plk-1 plays key roles in cell cycle by controlling mitotic entry, regulation at G2/M checkpoint, coordination of centrosomes, spindle assembly

regulation and segregation of chromosome. The overexpression of Plk-1 is a common cause of several caner including breast (Weib and Efferth, 2012). Inhibition of Plk-1 reduces proliferation of cancerous cells and stops cancer progression. Chemotherapy fails many time due to drug resistance of cancer cells and many studies has revealed that targeting Plk-1 can overcome this problem (Liu *et al.*, 2017).

***C. Exchange factor directly activated by cAMP***

Cyclin AMP is a second messenger involved in many process like growth differentiation, hormonal, neuronal, immunological regulation. Exchange factor directly activated by cAMP (Epac) is a cAMP target that regulates PKA independent signal transduction. They work as a guanine-nucleotide exchange factor (GEFs) for two G proteins, Rap1 and Rap2. It is involved in the progression of several types of cancer (Kumar *et al.*, 2017).

***D. N-acetyltransferase 2 (NAT2)***

N- acetyltransferase 2 (arylamine N-acetyltransferase) is an enzyme encoded by *NAT2* gene in humans. The Enzyme produced by *NAT-2* gene activates and deactivates certain hydrazime and arylamine drugs. N-acetylation property of this enzyme varies in human population due to polymorphism in *NAT-2* gene which results in segregation of the human population into rapid, intermediate and slow acetylator population. Polymorpohism in this gene also leads to drug toxicity and high incidence of cancer. Expression of *NAT-2* is found to be elevated in colon and colorectal cancers (da-Salva *et al.*, 2011).

***E. Phosphoinositide-3-kinase***

The members of Phosphoinositide-3-kinase (PI3K) takes parts in several process of cell development like growth, proliferation, survival, motility. PI3K is divided into three family: class I PI3K, class II PI3K and class III PI3K of which class I PI3K is the most important and further subdivided into two types i.e. class IA, class IB PI3K. Class I PI3K on activation generates phospholipids which act as secondary messenger for multiple signalling processes involved in cell regulation. Levels of PI3K in cells are finely tuned by PTEn. The PTEn is tumour suppressor gene and function antagonistically to the PI3K. Mutation in PTEn results in uncontrolled signalling by PI3K and causes cancer. The presence of mutated class I PI3K p 110 α is found to be common in many cancers which makes kinases more active leading to deregulation of

signalling (Liu *et al.*, 2009).

***F. Human Androgen receptor***

Androgen receptor is a steroid hormone receptor that belongs to group of nuclear receptors. It binds to ligands of 5α-dihydrotestosterone (DHT) and testosterone starts sexual development and differentiation in male and also acts as a transcription factor to control expression of several genes. Normal prostate cell and prostate cancer cells require androgen receptor and androgen for their growth and survival. Growth of prostate cancer depends on ratio of cell proliferation and death wherein prostate cancer ratio of proliferation is higher than death. This ratio is regulated by androgen receptor. Androgen receptor inhibition or androgen suppression leads to decline in cell proliferation and are the main strategies followed in prostate cancer treatment (Heinleng and Chase, 2004).

***G. Dihydrofolate reductase (DHFR)***

Dihydrofolate reductase (DHFR) is one of three important enzymes involved in folate and thymidylate synthesis pathways. Thymidylate cycle provides substrate for DNA synthesis and repair. Inhibition of DHFR leads to tetrehydrofolate deficiency which blocks pathways of thymidylate, purines, and methionine. Reduced supply of purines inhibits growth of oncogenic cell. Thus, blocking of DHFR causes reduced supply of purines and is used as a target in colon cancer (Schweitzer *et al.*, 1990).

***H. Estrogen receptor***

Estrogen is a naturally produced steroid hormone and is responsible for the maintenance and development of female sexual phenotype. Estrogen receptors are activated by estrogen hormone and later they translocate to the nucleus and subsequently binds to DNA to regulate the expression of several genes. Malfunctioning of estrogen receptor due to mutation or other physiological damage leads to cancer like breast cancer. Estrogen receptor promotes growth of cancer cells by targeting expression of signaling components of the insulin-like growth factor system. Chemical inhibitors of estrogen receptor are widely adopted method for breast cancer treatment (Somar and Faqua, 2001).

**2.5.3. ROS and Diabetes**

Diabetes mellitus (DM) is an inflammatory and metabolic disease which affects

nearly 40 million of the world population. Diabetes is of two types viz. type one and type two. Type one diabetes is found in juveniles also known as insulin dependent diabetes. This situation occurs due to autoimmune reaction of body. The body attacks own pancreatic cell producing insulin by raising antibody against it. It cannot be cured and person has to depend on insulin. In type two diabetes pancreas produces insulin but the amount of insulin is less or body become resistant to insulin. It mainly occurs in liver muscles, fats, *etc.* (Salsali and Nuthan, 2006). Progression of diabetes can be slowed down by using inhibitors but cannot be stopped.

Level of ROS increases under diabetic condition (Bayhs and Therpe, 1999). In a hyperglycemic conditions ROS decrease expression of insulin gene and secretion of insulin. During conditions where glucose concentration is high, β-cells of pancreas express GLUT2 transporter which uptake excessive glucose. However, high level of ROS causes damage to β-cells which reduces level of GLUT2 transporte (Harmone *et al.*, 2005). Subsequently ROS reduces mRNA expression and insulin gene promoter action (Kaneto *et al.* 2005). Lipotoxicity is another factor that causes deterioration of pancreatic β-cells. Free fatty acids produce ROS which causes dysfunction of β-cells (Bikopoulos *et al.*, 2008). The dysfunction of β-cells can lead to insulin resistance. Insulin activates signaling pathway by binding to insulin receptors on cell which are subsequently phosphorylated (Kadowiri, 2000). Reactive oxygen species interacts with insulin and disturb some pathways which ultimately affects the GLUT4 translocation which is involved in regulation of body glucose (Evans, 2002).

#### *2.5.3.1 Molecular targets identified for diabetes*

#### *A. Fructose 1, 6-bisphosphatase*

Fructose 1, 6-bisphophatase (FBPase) is an enzyme which converts fructose 1, 6-bisphosphate into fructose 6-phosphate. It also plays a key role in the hibernation process of animals and insects. Overproduction of glucose leads to the medical condition known as hyperglycaemia. Endogenously glucose is produced through two ways: first by de novo through 3-carbon precursors via gluconeogenesis. Second, through the breakdown of glycogen storages via glycogenolysis. Gluconeogenesis is responsible for the overproduction of glucose in type II diabetic patients (Magnusson *et al.*, 1992). FBPase is an important rate controlling enzyme of gluconeogenesis pathway (Erion *et al.*, 2005). Overproduction of glucose can be controlled by blocking the gluconeogenesis pathway via FBPase inhibition (Poelje *et al.*, 2006).

***B. Human Glucokinase***

Glucokinase is an enzyme of kinase family that converts glucose to glucose-6-phosphate through phosphorylation. It is present in all cells in liver and pancreas of humans and most other vertebrates. It plays the key role of carbohydrate metabolism in each of this organ by acting as a glucose sensor. Glucokinase is hexokinase isozyme, homologously related to three hexokinases (Kawai *et al.*, 2005).

Diabetes mellitus type II mainly occurs due to incorrect glucose metabolism which results in the high level of glucose or abnormal absorption of glucose in the liver after a meal due to delayed inactivation of hepatic glucose and impaired conversion of glucose to glycogen. Mutation in glucokinase coding genes is one of the major reason in many of diabetic Mellitus type II cases. In the case of inactivating mutations, the affinity of the enzyme for glucose is reduced or glucokinase expression is compromised while in case of activating mutations the blood glucose level is declined. The decline in glucokinase level has not been observed yet in diabetes but a rise in the level of glucokinase is found to be associated with diabetes type II and hence inhibition of glucokinase is a promising approach to treat or prevent diabetes type II (Agius, 2009).

***C. Glycogen synthase kinase-3***

Glycogen synthase kinase-3 (GSK-3) is a serine-threonine kinase which phosphorylates and inhibits glycogen synthase. It has two isoforms GSK3A and GSK3B. GSK3B functions in energy regulation, neuron cell development and body pattern formation. GSK-3 is continuously activated in resting cells and inhibited by the endothelial growth factor, platelet-derived growth factor and insulin by phosphorylation at Ser21 and Ser9.

Glycogen synthase (GS) is allosterically regulated by different kinases and GSK-3 plays a key role in this regulation by phosphorylation at three specific residues. It reduces the affinity of GS for binding to glucose-6-phosphate. GSK-3 also phosphorylates insulin receptor substrate (IRS)-1, a starting molecule in an insulin signalling cascade that leads to impaired regulation of insulin and to diabetes (Nikoulina *et al.*, 2002).

***D. Pyruvate dehydrogenase kinase***

Pyruvate dehydrogenase kinase (PDK) is an enzyme that belongs to kinase family and has a role in the phosphorylation of pyruvate dehydrogenase using ATP.

Pyruvate dehydrogenase is part of Pyruvate dehydrogenase complex (PDC) along with dihydrolipoyl transferase and pyruvate decarboxylase. Under normal glucose level, PDC converts pyruvate to acetyl COA which further enters to Krebs cycle for energy production.

Activation and deactivation of PDC is a finely tuned process. PDKs inactivates PDC while Pyruvate dehydrogenase phosphatase (PDPs) activates it. PDKs are transcriptionally regulated by insulin. Transcriptional up-regulation of PDKs reduces PDC activity and conversion of pyruvate to acetyl COA stops. This causes over accumulation of glucose in cells and finally, hyperglycemia occurs (Lee, 2014). Inhibition of PDK can be helpful to reduce glucose level.

***E. Aldose reductase***

Aldose reductase (AR) is a member of the oxidoreductase family that catalyses the reduction of aldehydes and carbonyls. It is well known for the conversion of glucose to sorbitol through the polyol pathway.

A major proportion of glucose is converted to glucose-6-phosphate by hexokinase which enters in glycolytic pathway during the normoglycemic condition, while polyol pathway accounts for conversion of only 3 per cent glucose. During the hyperglycemic condition, 30 per cent glucose is metabolised through the polyol pathway which creates oxidative stress on cells. There are three mechanisms behind this oxidative stress. First mechanism, underhyperglycemic condition, 30 per cent of glucose is utilizsed in AR- dependent polyol pathway which causes depletion in NADPH and reduction in Glutathione (GSH) level. Second, $NAD^+$ is converted to NADH during the conversion of sorbitol to fructose by sorbitol dehydrogenase. This NADH is a substrate for NADP oxidase which results in the production of superoxide anions. Third, at the end of the polyol pathway, fructose-6-phosphate and 3-deoxyglucosone are produced. These are more potent glycation agents than glucose (Tang *et al.*, 2012). Reduction of glucose to sorbitol by AR is a rate-limiting step of this pathway and hence inhibition of AR is a promising way to control diabetes mellitus.

***F. Multidrug resistance protein 1***

Multidrug resistance protein 1 (MRP-1) also known as P-glycoprotein (P-gp) or cluster of differentiation 243 (CD-243) is an important ATP dependent efflux pump with large substrate specificity. It is mainly present on the cell membrane and pumps

out foreign substances. There are six types of multidrug resistance protein. MRP-1 was the first protein of this family to be identified and is associated with efflux of anticancer drugs (Lautier *et al.*, 1996).

Long term diabetic therapies fail due to resistance development against drug and mutations in MRP. Development of resistance in MRP is due to change in ATP-binding cassette (ABC). Thus, inhibition of MRP-1 is considered as therapeutically important strategy for targeting diabetes (Koehn *et al.*, 2008).

***G. Dipeptidyl peptidase-4 (DPP-4)***

DPP4 also known as CD26 or adenosine monoamine complexing protein 2 is a protein coded by *DPP4* gene in humans. DDP-4 is present on the surface of cells is involved in apoptosis, signal transduction, immune regulation of kidneys, skin, capillary, endothelium, plasma, intestines, liver, uterus, prostate, placenta, and body fluids. DPP-4 is serine exopeptidase which cleaves proline or alanine from the N-terminal domain of proteins (Barnett, 2006).

Diabetes type II is controlled by regulating the blood glucose level. Level of glucose is maintained by insulin. Insulin level is increased by 50-70 per cent due to oral administration of glucose as compared to intravenous administration. This increase is due to the action of gut-derived hormones, glucagon-like peptide 1 (GLP-1) and gastric inhibitory peptide (GIP) known as the incretin effect (Creutzfeldt, 2005). GLP-1 is more active than GIP in the stimulation of insulin secretion. GLP-1 leads to glucose-dependent insulin increase and inhibit glucagon secretion. It also increases gastric acid production to inhibit gastric emptying in the GI tract. Incretin lives for a short period of time due to their degradation by DPP-4. DPP-4 cleaves proline and alanine residue at the second position of these proteins. Proline and alanine are critical for the functioning of GLP-1 and GIP. This results in faulty insulin regulation which causes hyperglycaemia. DPP-4 inhibition causes prolonged effects of endogenously synthesized incretins (Wani *et al.*, 2008).

### 2.5.4. Role of ROS in arthritis

Arthritis is a kind of joint disorders which involves pain and inflammation in joints. There are two types of arthritis *viz.* Osteoarthritis and Rheumatoid arthritis. Type one arthritis is arising due to trauma, infection of joints or age. While type two, rheumatoid arthritis is an autoimmune disease that comes with long-lasting pain.

Inflammation due to rheumatoid arthritis mainly occurs in synovial joints leading to bone and cartilage damage.

Oxidative stress causes damage to DNA. ROS produces lipid peroxides due to which peroxy radicals are generated which damage cell membranes, cell-matrix and leads to the development of the arthrosclerosis (Hilchonand E-Gabalwy, 2004). Low-density lipoproteins are damaged by ROS through oxidation process which in turn upregulates the chemokines glycation end products that cause inflammation (Rincon and Escealante, 2003). Inflammation along with oxidative stress causes protein degradation via glyoxidation and leads to the production of glycation end products. This glycation occurs in a protein associated with rheumatoid arthritis (Newkirk *et al.*, 2003). Reactive oxygen species also causes structural and functional changes in molecules associated with orthogenesis of ROS. Similarly, neo-epitopes are produced due to oxidation of protein by ROS which results in autoimmune responses (Eggleron *et al.*, 2013). Reactive oxygen species interferes with signaling pathways like MAPKs, PIK3-Akt, NF-AB are causes alteration which leads to increase inflammation (Phyll *et al.*, 2017).

#### *2.5.4.1 Molecular targets identified for arthritis*

***A. Nitric oxide synthase***

Nitric oxide synthase (NOS) is an enzyme that catalyses the production of nitric oxide from amino acid L-arginine. It plays an important role in cell signalling and as a neurotransmitter. Three isoforms of NOS are identified inducible NOS (iNOS), neuronal NOS (nNOS) and endothelial NOS (eNOS).

Inducible NOS (iNOS) is involved in the regulation of immune response. It binds to calmodulin at different concentration and produces free NO as part of the immune defence system. Under oxidative environment induction of iNOS increases and it leads to overproduction of NO. Nitric oxide gets a chance to bind superoxide and peroxynitrite is formed. Expression of iNOS is also stimulated by high levels of proinflammatory factors like Interleukin-1, Interferon gamma and Tumour necrosis factor-α (Green *et al.*, 1994). The level of NO found to be high in synovial fluid of rheumatoid arthritis patients (Nagy *et al.*, 2010). Production of NO can be controlled by inhibiting NOS and thereby reduction in inflammation and hence NO is a prime target for treating inflammatory disease (Kobayashi, 2010).

### *B. Cyclooxygenase*

Cyclooxygenase (COX) or prostaglandin endoperoxide synthase (PTGS) is an important enzyme in the production of prostaglandins from arachidonic acid. It also produces prostanoids like thromboxane and prostacyclin from arachidonic acid. COX have two isozymes COX-1 and COX-2 both have similar molecular weight and 65 per cent similarity in amino acid composition.

COX-1 is constantly expressed in most of the tissue for the production of prostaglandins while COX-2 remains unexpressed during normal conditions. Level of COX-2 increases during inflammation (Elder and Paraskeva, 1999; Gately, 2000). Induction of COX-2 is reported to have a role in rheumatoid arthritis and osteoarthritis (Anderson *et al.*, 1996; Amin *et al.*, 1997). Thus, it was found that COX-2 inhibition can provide relief from the pain associated with arthritis. Overexpression of COX-2 is also reported to be associated with several types of cancer like prostate, ovary, blood, breast, thyroid, cervical, *etc.* (Sonwane *et al.*, 2011).

### *C. Vascular endothelial growth factor*

Vascular endothelial growth factor (VEGF) is a subfamily of platelet-derived growth factors and cytokine knot growth factors. It acts as a signal protein involved in vasculogenesis. It activates two receptors VEGFR-1 and VEGFR-2. These receptors regulate pathological as well as physiological angiogenesis. VEGFR-1 is involved in inflammation, atherosclerosis, tumour growth and arthritis (Shibuyam, 2006) while VEGFR-2 is associated with diabetic retinopathy and cancer. Level of expression was found to be higher for VEGFs and its ligands in synovial fluid of rheumatoid arthritis patients (Murakami *et al.*, 2006). Hence, it is considered a potential target for treating arthritis.

### *D. Glucocorticoid receptor*

Glucocorticoid receptor (GR) interacts with signalling pathways such as PI3K, JNK proteins and components of the T cell receptor (TCR) signalling complex. It thereby modulate pro-inflammatory gene expression. The GR regulates inflammation both by direct transcriptional action on target genes and indirectly by inhibiting transcriptional activities of transcriptional factors such as NF-kB, AP-1 or interferon regulatory factors (Herrero *et al.*, 2015). Thus GRs have been implicated in inflammatory diseases and cardiovascular diseases such as atherosclerosis and hypertension (Kadmiel and Cidlowski, 2013).

### 2.5.5. ROS and Alzheimer

Alzheimer's is one of the most affecting neurodegenerative disorder among elder population of the world. Alzheimer's is characterized by neuronal and synaptic loss amyloid beta protein deposition and brain atrophy (Lemere and Masliah, 2010). It affects memory and vision. Alzheimer's occur due to genetic and environmental factors (Joseph *et al.*, 2003). The dysfunctioning of mitochondria as well as poor antioxidant regulation are primary reasons for occurrence of Alzheimer's. Free radicals like hydrogen peroxide, nitrous oxide, super oxide anion and hydroxyl ion causes neurodegradation in Alzheimer's disease (Xie *et al.*, 2002). Biometals like zinc iron and copper has a key role in neurodegradation (Barhanm *et al.*, 2003).

Oxidation of membrane phospholipid of brain cells by free radicals is a major contributor to Alzheimer disease pathogenesis (Baldeiras *et al.*, 2008). Reactive oxygen species also changes neuronal lipid molecule leads to alterations in fluidity, permeability, and transport. Antioxidants play major role in controlling excessive ROS and protect cell at some extent.

#### *2.5.5.1 Molecular targets identified for Alzheimer's*

***A. Human beta-secretase***

Human beta-secretase (BACE) is an aspartic protease, which is involved in the synthesis of peripheral nerve. It is also known as beta site amyloid precursor protein cleaving enzyme 1/membrane associated aspartic protease 2/memapsin-2, aspartyl proteases-2/Beta-site APP cleaving enzyme. It is coded by *BACE-1* gene in humans (William *et al.*, 2006).

Accumulation of extracellular deposits called amyloid plaques in the cerebral region causes Alzheimer's disease. This plaque is composed of amyloid β peptides (Aβ) containing of 38-43 amino acid. Aβ is primarily produced by neurons through sequential proteolysis of type 1 membrane protein APP. First, β-secretase cleaves APP to produce C99 (membrane bound C-terminal fragment). Later, C99 is cut by gamma-secretase and yields Aβ. Third protease α-secretase can cleave at sites of Aβ within APP protein. These Aβ peptides are toxic. The enzymes like β-secretase, gamma secretase play a key role in production, inhibition and regulation of β-secratase. Hence, it is considered as good therapeutic target for Alzheimer's diseases treatment (Yan and Vassar, 2014).

***B. Tau protein***

Tau proteins are found abundantly in neurons of the central nervous system (CNS). Tau protein stabilizes microtubules. It is coded by a single gene *MAPT* (microtubule-associated protein tau) located on chromosome No. 7 and is produced through alternate splicing of the gene (Goedert *et al.*, 1998).

Tau regulates the assembly and structural stability of microtubules in healthy cells under normal physiological conditions. When tau becomes hyperphosphorylated, it causes disassembly of microtubules and Tau proteins aggregates leading to formation of neurofibrillary degeneration and forms a helical like structure called tangles leading to neurofibrillary degeneration (Alonso *et al.*, 1997). Phosphorylation occurs at different serine residues. Tubulins which normally carries nutrients for nerve cells failed to do so after tangling and consequently neurons start dyeing results into memory loss and several other problems associated with Alzheimer's (Medeiros *et al.*, 2011). Inhibition of Tau proteins aggregation can be a promising strategy to control Alzheimer's.

***C. Acetylcholinesterase***

Acetylcholinesterase (AChE) is the enzyme that catalyzes the breakdown of acetylcholine and some choline esters that functions as neurotransmitter.
cetylcholinesterase (AChE) is the primary cholinesterase in humans. It reacts on neurotransmitters like acetylcholine (ACh) and other choline esterase. It is involved with several processes memory storage, acquisition, encoding, consolidation, retrieval of memory, extinction (Talita *et al.*, 2016). The ACh also regulates the sleep cycle as well as organizes a neuronal response to the visual cortex (Paez-Gonzalez *et al.*, 2014).

Signalling between two neurons is mediated by the release of neurotransmitters like Ach. Transmitter neuron releases Ach which binds to the receptor of Ach on receiving neurons. The AChE acts on ACh and breaks it into acetic acid and choline and maintains an optimal amount of neurotransmitters. Choline goes back to presynaptic cholinergic neurons. In Alzheimer's patients, neurotransmitters are released in low amount and reduction of ACh by AchE stops the signalling between two cells. AChE inhibitors bind to AChE and prevent it from the action on ACh results in smooth signalling between neurons. However, inhibitors used against AChE are effective only for 6-12 months. They are not effective to slow down Alzheimer's progression and consumption of these inhibitors comes with the side effect (Raina *et al.*, 2008). So use

of active natural compound can solve this problem and can be considered for drug discovery.

***D. Butyrylcholinesterase***

Butyrylcholinesterase (BChE) is a nonspecific cholinesterase that hydrolyses a variety of cholinesters. It is coded by the *BCHE* gene in humans. It is produced in the liver and secreted to the blood plasma. The BChE is 50 per cent similar to AChE in amino acid composition. However, during the progression of Alzheimer's, 45 per cent of AchE is lost while there is 90 per cent increase in levels of BChE (Wright *et al.*, 1993). The BChE catalyses the reduction of ACh into choline and acetic acid. It plays a key role in Aβ aggregation in the initial stages of senile plaque formation and it amplifies the toxicity of β-amyloid. The treatment of Alzheimer's includes not only possible through inhibition of AChE but optimal suppression of BChE is also important (Grieg *et al.*, 2002).

## 2.8 *IN SILICO* DOCKING STUDIES IN CURRY LEAF

Murugan *et al.* (2013) studied antiaflatoxigenic potential of curry leaf as food additive by molecular interaction and *in vitro* methods. Homology modelling was done for Ver1 protein of *Aspargillus flavus* using Discovery Studio 2.5. Among the phytochemicals of curry leaf 13 bioactive compound showed interaction with target Ver1. Isophytol showed good docking score about 72.925 and with -8.079 kcal/mol of internal energy and distance of 2.029 Å. Binding of phytocompounds to target may causes interference in aflatoxin synthesis at transcriptional level.

Manimekalai *et al.* (2015) performed *in silico* docking of carbazole alkaloids from curry leaf against phosphoprotein pohosphatase (PP2A). Seven carbazole alkaloids showded interaction with target (1WAO). Among all, 1-formyl 3-methoxy 6 methyl carbazole had shown good docking score (docking energy of -7.56 kcal/mol, interaction via two amino acid viz. Tyr78, Tyr313). A compound, 6, 7-demethoxy 1-hydroxy carbazole formed highest number of hydrogen bonds (3) while o-methyl murrayanine formed single hydrogen bond. Binding of phytocompounds to PP2A activates it and causes reduction in phosphorylation of tau protein which offers a new way of treating Alzheimer's.

Ismail *et al.* (2016) demonstrated the inhibition of the proteasome by alkaloids of *Murraya koenigii*, which lead to cancer cell death. They docked 26 carbazole alkaloids of *Murraya koenigii* with the beta subunits of the catalytic 20S proteasome using



44

Accelrys Discovery Studio (ADS) v2.1. Three out of 26 compounds showed interaction with 20S proteasome. Murrayacine interacted with β5 subunit of target via Thr57, Ile35 and Glu36 having distance more than 3 Å with docking energy of -76.66845 kcal/mol. Koeinine interacted with β2 subunit of target via Ala49, Ala32 and Lys33 with binding energy of -84.9547 kcal/mol. Similarly, Mahanine interacted with β2 subunit of target via Gly208, Tyr210 and Val212 with binding energy of -84.9547 kcal/mol.

Salomi *et al.* (2016) performed comparative *in silico* docking of superoxide dismutase 1 (SOD1) against natural and synthetic antioxidants using an AutoDock software. Two compounds of curry leaf O-methylmahanine, O-methylmurrayamine and synthetic antioxidant Butylated Hydroxytoluene (BHT) interacted with target (SOD1) via Thr88, Lys32, and Asn86 respectively with binding energy of -6.61, -6.69 and -4.56 kcal/mol. Binding energies of curry leaf compounds were stronger than BHT.

Salomi *et al.* (2016) carried out *in silico* docking analysis of natural antioxidant from *Murraya koenigii* and butylated hydroxyanisole. Three compounds (Koenimbine and mahanine and standard antioxidantBHA) interacted with target (Superoxide dismutase 3). Koenimbine bound to the target via His153 with docking energy of -4.89 kcal/mol while Mahanimbine bound to the target via Phe84 with docking energy of -5.33 kcal/mol. The binding energy for BHA was -3.85 kcal/mol which was less among three. and interacted via Ala32.

Carbazole alkaloids from *Murraya koenigii* possess wound healing property. The Monocyte/Macrophage chemoattractant protein-1 (MCP-1) is protein involved in formation of mature vessels and collateral arteries. It plays an important role in extravascular wound healing. MCP-1 is also associated with formation of new blood vessels and hence wound healing can be enhanced by increasing activity of MCP-1. Salomi *et al.* (2017) studied interaction of curry leaf phytocompounds to MCP-1 and found that five carbazole alkaloids were interacting with MCP-1. Among five Mahanimbinol recorded best docking score by forming four hydrogen bonds viz. Pro-8, Thr-10, Thr-32, Ser-33 and docking energy of -7.77 kcal/mol.

Shabnashmi and Cynthia (2017) studied *in vitro* and *in silico* inhibitory activity of *M. koenigii* against streptococcus mutant. Molecular docking was performed using AutoDock. Three curry leaf compounds viz. Bismurrayafoline A, Murrayacine and Murrayazoin were docked against target proteins from *streptococcus* mutant (AlkD2, SMU1763C, dextran glucosidase). Bismurrayafoline A interacted with all the target proteins with docking energies of -6.44, -7.29 and -8.24 kcal/mol respectively.

Similarly, Murrayacine interacted with all target proteins while Murrayazoin interacted with two target poteins (SMU1763C, dextran glucosidase).

Rameen *et al.* (2018) tested the binding efficiency of five compounds present in the *Murraya koenigii* with the thirteen viral proteins targets (dengue and Marburg disease) through *in silico* methods. Molecular docking result showed the highest binding affinity of 2-Phenyl-4 Quinoline carboxamide with the proteins and they also predicted the binding site amino acid residues and the type of hydrogen bonding.

Bhavya *et al.* (2018) conducted *in silico* molecular docking in *Murraya koenigii* for comparative study of both two structural and five non-structural proteins for dengue virus along with seven structural and two non-structural proteins for Swine flu. The ligands were taken from GCMS analysis of *Murraya koenigii*. Interaction of five compounds of curry leaf with 14 targets of virus studied. The compound 1, 5-Diformyl-2,6-Dimethoxy-Anthracene interacted with all 14 target proteins with high binding energy. The amino acids present in binding site and hydrogen bond type were also predicted.

Ahmed *et al.* (2019) carried out molecular docking studies to study the interaction of phytocheimcals in *Murraya koenigii* leaf. Alkaloids were found to be main constituents of extract after preliminary investigation. Three carbazole alkaloids identified from leaves were Mohanimbin, Koenimbine and Euchristine B. Molecular docking studies showed that most of the carbazole alkaloids have interaction, especially Euchristine B interacted with alpha-glucosidase with very good H-bond interaction (-5.326 kcal/mol) and Koenimbine interacted good with human aldose reductase (-2.422 kcal/mol) and with alpha-glucosidase (-3.133 kcal/mol).

# *Materials and Methods*

# 3. Materials and Methods

The study entitled "Characterization of antioxidant fractions in curry leaf (*Murraya koenigii* L.) and molecular docking of selected bioactive compounds" was carried out at the Centre for Plant Biotechnology and Molecular Biology (CPBMB) and Distributed Information Centre (DIC) College of Horticulture, Kerala Agricultural University during 2017-2019. The objectives of the study were to characterize antioxidant fractions in curry leaf through *in vitro* assays and to identify the most potent bioactive compound through LC-MS/MS and molecular docking analyses.

## 3.1 MATERIALS

### 3.1.1 Plant materials

Curry leaf variety Suvasini was used for this study. Centre for Plant Biotechnology and Molecular Biology has a standard regeneration protocol for micropropagation of curry leaf. Tissue culture plants of variety Suvasini raised at experimental field of CPBMB was used to collect leaves for the experiment.

### 3.1.2 Laboratory chemicals and glasswares

The analytical grade acetone used for oleoresin extraction and chemicals used for column chromatography like Hexane, ethyl acetate were procured from SISCO Research Laboratories Pvt. Ltd. Diphenyl-1-picrylhydrazyl (DPPH) used for antioxidant assay was also procured from SISCO Research Laboratories Pvt. Ltd. Butylated hydroxyanisole, a standard antioxidant and Thin layer chromatography plates were obtained from Sigma Aldrich Pvt. Ltd. Methanol was obtained from Himedia Pvt. Ltd. Borosilicate glass wares and plastic wares used were procured from Borosil, Riveria and Tarsons Products Pvt. Ltd.

### 3.1.3 Equipment and machinery

For the present study, equipment available at the Centre for Plant Biotechnology and Molecular Biology (CPBMB) were used. Computer facility and software support were provided by Distributed Information Centre (DIC) of College of Horticulture. Soxhlet apparatus was used for the oleoresin extraction. Antioxidant assay was carried out using U.V. spectrophotometer of Thermo Scientific (Genesys). LC-MS/MS analysis was done at Bombay in Sophisticated Analytical Instrument Facility of Indian Institute of Technology. Accelry Discovery Studio 4.0 (USA) software, available at DIC of College of Horticulture was used for docking studies.

3.2 METHODS

### 3.2.1 Collection of leaves

Leaves were collected from five year old curry leaf plants available in the CPBMB demonstration field. Collection of leaves was done on basis of maturity. Two types of leaves were collected. First was medium mature leaves with light green colour and second was mature leaves with dark green colour.

### 3.2.2 Drying of leaves

Leaves were plucked from branches and washed. After wiping with sterile cloth, one kilogram leaves were weighed and spread on paper for shade drying. Leaves were allowed to dry for almost 20 days until they break easily.

### 3.2.3 Powdering of sample

After 20 days, dehydrated leaves were ground into fine powder using grinder. The powder was kept in air tight container and was refrigerated to prevent loss of compounds.

### 3.2.4 Extraction of oleoresin from curry leaf powder

The extraction of oleoresin from curry leaf powder was done using Soxhlet apparatus as per AOAC (1980). Soxhlet apparatus works on principle of solvent extraction.

#### 3.2.4.1 Procedure

Ten gram of curry leaf powder was weighed and packed in a coarse filter paper. This was placed in the extraction chamber of apparatus. Extraction was carried out in the apparatus by using 100 per cent acetone for nine hours till the solvent becomes colourless. After extraction, the extract was transferred to a pre-weighed beaker. The beaker was kept open for evaporation to remove final traces of acetone and final weight of beaker recorded. The recovery of oleoresin (%) was calculated suing the following formula:

$$\frac{Weight\ of\ oleoresin}{weight\ of\ curry\ leaf\ powder} \times 100$$

### 3.2.5 Antioxidant assay

The extracted oleoresin was subjected to antioxidant assay to monitor its potential to scavenge the reactive oxygen species (ROS). In the present study

antioxidant assay was performed using DPPH system using UV spectrohototmeter (Shimada *et al.*, 1992).

### *3.2.5.1 Procedure of assay*

The experiment was conducted with three replications. A solution of concentration 50 ppm was prepared from oleoresin of medium mature and mature leaves along with Butylated Hydroxyanisole (BHA) using methanol. In test tubes, 1625 µL of sample (extract/standard/control) was taken and to that 375 µL of methanolic solution of DPPH (120 ppm) was added. Test tubes were kept in dark and incubated at $27^0$C for 20 minutes. Baseline correction was done using methanol and optical density was recorded at 517 nm. Radical scavenging activity was articulated in terms of percent inhibition and activity was calculated by the following formula:

$$\text{Radical scavenging activity (\%)} = \frac{Control\ O.D. - Sample\ O.D.}{Control\ O.D.} X100$$

## 3.2.6 Separation of antioxidant fractions from curry leaf by column chromatography

Oleoresin with maximum radical scavenging activity was used for further studies. It was subjected to silica gel column chromatography for fraction separation (Rao *et al.*, 2007).

### *3.2.6.1 Packing of column*

Glass column of 720 mm X 15 mm was used. Column was pre-equilibrated with 100 per cent hexane. Silica gel (200-400) 25 gram was weighed and kept in a hot air oven at $100^0$C for 3 hrs. Slurry of silica gel was prepared by adding 50 ml of 100 per cent hexane. This slurry was carefully loaded into column without air bubble. Column was washed 2-3 times with 100 per cent hexane.

### *3.2.6.2 Preparation of sample*

Two gram of the oleoresin extracted from mature leaves powder was mixed with 20 ml of hexane. Solution was stirred well.

### *3.2.6.3 Preparation of solvent system*

Solvent system for fraction separation by column chromatography prepared using solvents of varying polarity *viz.* hexane (nonpolar) and ethyl acetate (polar) in different proportions such as 100:0, 80:20, 60:40, 40:60, 20:80, 0:100 (hexane: ethyl acetate).

*3.2.6.4 Procedure*

Samples dissolved in hexane were loaded carefully over top of packed column. Care was taken not to disturb layer of silica. Sample was allowed to move into column. Afterwards, column was filled with 75 ml of 100 per cent hexane and fraction was collected using glass beakers. Further, elution was done by adding 75 ml of 80:20, 60:40, 40:60, 20:80 and 0:100 (hexane: ethyl acetate) respectively. Each fraction was collected separately. Afterwards, those fractions were transferred to pre-weighed beakers and kept open for evaporation to remove final traces of the solvents. Then, DPPH assay was performed to find out the fraction with maximum antioxidant activity. This experiment was done with three replications.

**3.2.7. Sub-fractionation of selected fraction**

Fraction with highest antioxidant activity was subjected to sub-fractionation. The fraction was loaded into silica column and subjected to column chromatography. Fractions were collected at 5 minute interval. Total 47 sub-fractions were collected. The collected sub-fractions were transferred to pre-weighed beakers and solvent was evaporated. DPPH assay was performed with 47 sub-fractions to find out the sub-fraction with maximum antioxidant activity.

**3.2.8. Identification of compound through LC-MS/MS**

The sub-fractions with highest antioxidant activity and main fraction were dissolved in 2 ml of methanol and were sent to Sophisticated Analytical Instrument Facility of Indian Institute of technology, Bombay for LC-MS/MS analysis. Samples were analysed with Agilent G6550A with triple quadrupole mass spectrophotometer. Samples were mixed with water:acetonitrile in the ratio 95:5 and 3 µl of sample was injected to machine. Electrospray ionization with positive polarity (ES+) was given at 3500 V capacity voltage, 1000 V nozzle voltage, gas was provided at 13 L/min with source temperature 250° C.

**3.2.9. Molecular docking studies of selected bioactive compounds**

Molecular docking is a method of targeting small molecules (ligands) into biological targets either to activate or inhibit them. Biological targets can be proteins, enzymes, receptors or ion channels. Ligands are natural or synthetic compounds binds to the active sites of targets. In the present study, various biologically active compounds in curry leaf identified through LC-MS/MS analysis were taken as ligands and docked

against targets for cancer, diabetes, arthritis and Alzheimer's and effectiveness of curry leaf phytocompounds was compared with commercially available drugs for cancer, diabetes, arthritis and Alzheimer'.

### *3.2.9.1. Retrieval of structure of curry leaf phytocompounds and commercially available drugs*

The Structures of compounds identified through LC-MS/MS and selected commercial drugs (Table 1) retrieved from Pubchem and Chemspider database (Fig.1). Compound names were entered in search box of databases and 3D structure was downloaded in '.sdf' format. Structure was drawn for unreported compounds using ACD Chemsketch software. Later those structures were converted into 3D and saved in .mol format.

**Table 1: Commercially available drugs for cancer, diabetes, arthritis and Alzheimer's**

| Sl. No. | Disease | Commercially available drugs |
|---|---|---|
| 1 | Cancer | Fulvestrant, Ribociclb, Diphenylamine, NSC-54767, Tamoxifen, Ferretinide, Trimetrexate |
| 2 | Diabetes | Lithium carbonate, Dichloroacetic acid, Epalrestat, Technetium Tc99m medronate, Apigenin, Myricitin |
| 3 | Arthritis | Apigenin, Diclofenac, Rorafenib, Dexamethasone |
| 4 | Alzheimer's | Verbecestat, Galanthamine, Rivastigmine |

### 3.2.9.2 Selection of target for different diseases

Targets for cancer, diabetes, arthritis and Alzheimer's were identified through literature survey. Name of target, type of disease and scientist reported were tabulated in Table 2


**Fig. 1: Homepage of Pubchem**

**Table 2: Targets selected for different diseases**

| Sl. No. | Disease | | Target Compound | PDB I.D. | References |
|---|---|---|---|---|---|
| 1 | Cancer | 1 | 17 beta-HSD (Breast Cancer) | 1FDT | Gunnarsson *et al.*, 2005 |
| | | 2 | Polo-like kinase 1 (Breast Cancer) | 3KB7 | Mathew *et al.*, 2017 |
| | | 3 | Exchange protein directly activated by CAMP (Pancreatic cancer) | 3CF6 | Almaharia *et al.*, 2013 |
| | | 4 | Nat -2 receptor (All type of cancer) | 2PFR | PDTD |
| | | 5 | Phosphoinositide-3 kinase (All type of cancer) | 1E8W | PDTD |
| | | 6 | Human androgen receptor (Prostate Cancer) | 1E3G | Singh *et al.*, 2017 |
| | | 7 | Dihydropholate reductase (Colon cancer) | 4DFR | Al-harbi *et al.*, 2015 |
| | | 8 | Human estrogen receptor ligand-binding domain (Breast Cancer) | 1ERR | Roy, 2016 |
| 2. | Diabetes | 1 | Fructose-1, 6-bisphosphatase | 2JJK | Mahendrana *et al.*, 2014 |
| | | 2 | Human glucokinase | 1V4S | Balamurugan *et al.*, 2012 |
| | | 3 | Glycogen synthase kinase | 1Q5K | Bustanji *et al.*, 2008 |
| | | 4 | Pyruvate dehydrogenase kinase | 4MP2 | Natrajan *et al.*, 2015 |
| | | 5 | Human aldose reductase | 3G5E | Natrajan *et al.*, 2015 |
| | | 6 | Human multidrug resistance protein | 2CBZ | Balamurugan *et al.*, 2012 |
| | | 7 | Human dipeptidyl peptidase IV | 1X70 | Roy, 2016 |
| 3. | Arthritis | 1 | Nitric oxide synthase | 4NOS | Fischman *et al.*, 1999 |
| | | 2 | Cyclooxygenase-2 | 4COX | Kurumbail *et al.*, 1996 |
| | | 3 | Vascular endothelial growth factor receptor | 3HNG | Roy, 2016 |
| | | 4 | Glucocorticoid receptor | 1M2Z | Bledsoe *et al.*, 2002 |
| 4. | Alzheimer's | 1 | Human beta Secratase 1 | 2XFJ | Atlam *et al.*, 2018 |
| | | 2 | Tau protein kinase 1 | 1J1B | Barai *et al.*, 2018 |
| | | 3 | Human acetylcholineestrease | 4BDT | Larik *et al.*, 2018 |
| | | 4 | Human butyrylcholinesterase | 4BDS | Monterio *et al.*, 2018 |

### *3.2.9.3. Retrieval of targets structure*

Structure for targets were retrieved from Brookhaven National Laboratory's database on protein structures. Protein Data Bank (PDB) homepage was opened (url) and protein name/PDB ID was entered in the search box (Fig. 2). Resulting 3D


**Fig. 2: Homepage of Protein Data Bank (PDB)**

structure was saved in PDB file (Text). X-ray diffraction method and resolution power were taken into consideration during the retrieval of targets.

#### *3.2.9.4 Predication of the active sites for targets*

Active site is the ligand binding region of the target. Prediction of target is a crucial step in docking process. Discovery studio 4.0 'Receptor cavity and Prediction tool' was used to predict active site of the target. Prediction was based on number of active amino acid present in the binding site. Binding site of ligand was corrected and analysed by tools in Discovery studio 4.0.

#### *3.2.9.5 Molecular docking and analyses*

Discovery Studio 4.0'S "CDOCKER" protocol was followed for molecular docking between the target and identified phytocompounds in curry leaf. Bioactivity of compounds was assessed as per above protocol. Biologically active inhibitors for targets related to various diseases were identified.

#### *3.2.8.5.1 Preparation of protein*

Retrieved protein was prepared by using protein preparation wizard of "Accelry Discovery studio 4.0" (USA). Extra chain of target protein, internal bound ligand, heteroatoms and crystallographic water molecules were removed and chemistry of protein was corrected using hydrogen atoms. CHARMm force field was used for energy minimization of protein. The steps followed were as follows. Open DS 4.0-> Click on file-> Click open-> Add a protein molecule-> Click on macromolecules-> Click prepare protein-> click on run on the new window of the protocol.

#### *3.2.8.5.2 Preparation of ligand*

Ligands were prepared by removing duplicates, adding hydrogen atom, enumerating tautomers/isomers and energy optimization using CHARMm (Chemistry at Harvard Macromolecular Mechanics) force field (Brooks *et al.*, 1983 and Brooks *et al.*, 2009). The steps followed were as follows: Open DS 4.0-> Click on file-> Click Open-> Add a ligand-> Click on small molecule-> Click on prepare ligands-> Click Run on the new window of the protocol.

#### *3.2.8.5.3 Filtration of ligands*

Ligands were filtered as per Lipinski-Veber's protocol to assure drug likeness and bioavailability of drug. To pass this criteria compound should be of less than 500

daltons molecular weight. Hydrogen bond donors should not surpass 5, while maximum 10 hydrogen bond acceptor are accepted, and log P values should not exceed than 5 (Lipinski *et al.*, 2001 and Lipinski, 2004). As per Veber's protocol polar surface value should be less than 140 Å, number of rotatable bonds should not exceed than 10 (Veber *et al.*, 2002). The steps followed were as follows: Window of ligand preparation was opened from recent jobs and "Filter by Lipinski and Veber rules" was selected from protocols under small molecules and programme was run. Filtered ligands were used for molecular docking.

***3.2.8.5.4 Protein- ligand docking***

Prepared target protein and identified compounds form curry leaf were docked to find out best pose and to know binding affinity of compounds against each target molecule using CDOCKER of D.S. 4.0 (Wu *et al.*, 2003). Docking was targeted to predicted active sites of target. Docking simulation was done using molecular dynamics. Maximum ten poses were allowed to be analysed based on minimum difference between C-DOCKER and C-DOCKER interaction energy to find out best pose. Scoring function was based on calculation of binding energies. The calculations were performed first on target and later on ligand and finally on the complex. The difference of energy was then calculated by using following equation: $\Delta E = E_{complex} - E_{ligand} - E_{protein}$ ($\Delta E$ is the ligand binding energy). Steps followed were as follows: Open DS 4.0-> click on Receptor ligand interactions-> Click on Dock ligands-> select CDOCKER protocol-> select input receptor as the visible prepared protein structure-> select input ligands as the visible filtered compound structure-> Click on Run.

***3.5.8.5.5 ADME/T analysis***

ADME/T refers to absorption, distribution, metabolism, excretion and toxicity. These are pharmacokinetic (PK) properties to assure drug-likeness. Docked ligands were subjected to ADME Descriptor algorithm of Accelrys Discovery studio 4.0 (USA). Pharmacokinetic properties like Aq. solubility, Human Intestinal Absorption, Blood-Brain-Barrier (BBB) penetration, cytochrome P450 inhibition and Hepatotoxicity levels were estimated for docked ligands. Standard levels of this parameters are mentioned in Table 3.

**Table 3: Standard level of ADME/T descriptor**

| Sl. No. | ADME/T Descriptors | Rankings | | | | | |
|---|---|---|---|---|---|---|---|
| 1. | Solubility level | 0-Extremely low | 1-Very low | 2-Low | 3-Good | 4-Optimal | 5-Too soluble |
| 2. | BBB level | 0-Very High penetrant | 1-High | 2-Medium | 3-Low | 4-Very low | |
| 3. | CYP2D6 Prediction | False-Non Inhibitor | True-Inhibitor | | | | |
| 4. | Hepatotoxic Prediction | False-Nontoxic (0) | True-Toxic (1) | | | | |
| 5. | Absorption level | 0-Good | 1-Moderate | 2-Poor | 3-Very poor | | |

# Results

# 4. Results

The results of the study entitled 'Characterization of antioxidant fractions in curry leaf (*Murraya koenigii* L.) and molecular docking of selected bioactive compounds' are presented in this chapter under different headings.

## 4.1 EVALUATION OF ANTIOXIDANT FRACTIONS FROM CURRY LEAF

### 4.1.1 Extraction of oleoresin

Oleoresin was extracted from shade dried medium mature and mature leaves of curry leaf. Yield of oleoresin is presented in Table 4. Variation was observed in yield of oleoresin from mature and medium mature leaves. Mature leaves recorded highest oleoresin yield of 9.16 per cent while recovery of oleoresin was less in medium mature leaves with 7.87 per cent.

### 4.1.2 Antioxidant assay

Oleoresin extracted from medium mature and mature leaves were subjected to DPPH assay to know the potential of extract to scavenge radicals of reactive oxygen species (ROS). Percent inhibition of ROS is presented in Table 5. Butylated hydroxyanisole, the standard used recorded the highest per cent inhibition with 91.14 followed by oleoresin extracted from mature leaves with 85.19 per cent and oleoresin extracted from medium mature leaves with 83.30 per cent. Oleoresin extracted from mature leaves was found better than oleoresin extracted from medium mature leaves and it was used for further studies.

### 4.1.3 Separation of antioxidant fractions from curry leaf by column chromatography

#### *4.1.3.1 Separation of oleoresin*

Oleoresin was separated by column chromatography using solvent system composed of hexane and ethyl acetate in different proportion such as 100:0, 80:20, 60:40, 40:60, 20:80, 0:100. Solvent was evaporated from fractions and yield of extract in each fraction was recorded (Table 6). Yield of fractions varied in the range of 50.6 mg to 706.4 mg. fraction extracted using 60:40 (hexane: ethyl acetate) recorded highest yield of 706.4 mg while the lowest was recorded in fraction extracted using 100 per cent ethyl acetate (50.6 mg).

**Table 4: Effect of maturity of leaves on oleoresin yield in curry leaf**

| Sl. No. | Maturity of leaves | Yield of oleoresin (%) |
|---|---|---|
| 1 | Medium mature | 7.87 |
| 2 | Mature | 9.16 |

**Table 5: Effect of maturity of leaves on antioxidant property in curry leaf**

| Sl. No. | Treatment | R1 (%) | R2 (%) | R3 (%) | Mean (%) |
|---|---|---|---|---|---|
| 1 | Standard (BHA) | 91.25 | 91.30 | 90.89 | 91.14 |
| 2 | T1 | 84.00 | 83.54 | 82.38 | 83.30 |
| 3 | T2 | 84.66 | 86.33 | 84.59 | 85.19 |

T1: Medium mature leaves; T2: Mature leaves

**Table 6: Weight of extract in different fractions separated by column chromatography**

| Sl. No. | Fraction | Weight of extract (mg) |
|---|---|---|
| 1 | 100:0 (Hexane: Ethyl acetate) | 57.1 |
| 2 | 80:20 (Hexane: Ethyl acetate) | 117.7 |
| 3 | 60:40 (Hexane: Ethyl acetate) | 706.4 |
| 4 | 40:60 (Hexane: Ethyl acetate) | 470.3 |
| 5 | 20:80 (Hexane: Ethyl acetate) | 63.2 |
| 6 | 0:100 (Hexane: Ethyl acetate) | 50.6 |

#### *4.1.3.2 Antioxidant assay*

Radical scavenging potential of each fraction was determined using DPPH system. There was significant variation in per cent inhibition of DPPH activity in all fractions (Table 7). Inhibition of DPPH varies from 39.68 to 88.68 per cent in all the fractions. The fraction (60:40 hexane: ethyl acetate) recorded the highest percent of DPPH inhibition (88.68%) while lowest inhibition of DPPH (39.68%) was recorded in fraction (0:100). Standard BHA being a pure preparation, recorded highest inhibition of DPPH (91.17%) and was significantly superior to other treatments. The Fraction (60:40 hexane: ethyl acetate) possessing maximum potential of inhibition of DPPH was selected as best fraction and used for further studies on sub-fractionation.

### 4.1.4 Sub-fractionation of fraction (60:40 hexane: ethyl acetate) and antioxidant assay

The whole fraction (60:40 hexane: ethyl acetate) was subjected to sub-fractionation at five minutes internal using column chromatography. Total 47 sub-fractions were collected and evaporated. All sub-fraction were subjected to antioxidant assay using DPPH system. Details of DPPH inhibition percentage is shown in Table 8. The DPPH inhibition percentage varied in different fractions. Fifty seven per cent of sub-fractions showed inhibition of DPPH activity in between 60 to 80 per cent while 17 per cent of sub-fractions showed less than 50 per cent DPPH inhibition activity. Twenty seven per cent sub-fractions exhibited high percentage of DPPH inhibition (<80%), of which, sub-fraction No. 28 collected at 140 min. showed maximum inhibition of 91.51 per cent. Five sub-fractions, possessing maximum antioxidant activities viz. fraction No. $26^{th}$, $28^{th}$, $34^{th}$, $38^{th}$ and $40^{th}$ collected at 130, 140, 170, 190 and 200 min respectively with 91.08, 91.51, 91.08, 89.53 and 89.53 per cent inhibition of DPPH respectively were selected for LC-MS/MS analysis. The DPPH activity of sub-fractions $26^{th}$, $28^{th}$, and $34^{th}$ was as par. The DPPH inhibition activity of sub-fractions $26^{th}$, $28^{th}$ and $34^{th}$ was on par.

### 4.1.5 LC-MS/MS analysis

#### *4.1.5.1 Number of compounds identified through LC-MS/MS*

Analysis of whole fraction (60:40 Hexane: ethyl acetate) and five sub-fractions *viz.* $26^{th}$, $28^{th}$, $34^{th}$, $38^{th}$ and $40^{th}$ sub-fraction by LC-MS/MS was done by SAIF, IIT, Bombay. From each sample 100 compounds were detected. The presence of

**Table 7: Inhibition of DPPH for different fractions separated by silica gel column chromatography**

| Sl. No. | Type of Solvent | Inhibition of DPPH (%) |
|---|---|---|
| 1 | Standard | 91.147 |
| 2 | 100:0 (Hexane to ethyl acetate) | 46.770 |
| 3 | 80:20 (Hexane to ethyl acetate) | 69.527 |
| 4 | 60:40 (Hexane to ethyl acetate) | 88.680 |
| 5 | 40:60 (Hexane to ethyl acetate) | 85.140 |
| 6 | 20:80 (Hexane to ethyl acetate) | 65.920 |
| 7 | 0:100 (Hexane to ethyl acetate) | 39.680 |
| | | C.D. = 0.669 |

**Table 8: Inhibition of DPPH for different sub-fractions of 60:40 (hexane: ethyl acetate) separated by column chromatography**

| Sl. No. | Fraction No. | DPPH inhibition (%) | Sl. No. | Fraction No. | DPPH inhibition (%) |
|---|---|---|---|---|---|
| 1 | BHA (standard) | 91.895 | 25 | 24 | 85.585 |
| 2 | 1 | 50.390 | 26 | 25 | 74.885 |
| 3 | 2 | 11.705 | 27 | 26 | 91.080 |
| 4 | 3 | 13.680 | 28 | 27 | 78.560 |
| 5 | 4 | 79.455 | 29 | 28 | 91.510 |
| 6 | 5 | 61.300 | 30 | 29 | 77.480 |
| 7 | 6 | 76.405 | 31 | 30 | 75.675 |
| 8 | 7 | 50.840 | 32 | 31 | 62.215 |
| 9 | 8 | 63.515 | 33 | 32 | 68.545 |
| 10 | 9 | 17.245 | 34 | 33 | 78.105 |
| 11 | 10 | 43.605 | 35 | 34 | 91.080 |
| 12 | 11 | 55.310 | 36 | 35 | 73.530 |
| 13 | 12 | 64.655 | 37 | 36 | 85.970 |
| 14 | 13 | 69.450 | 38 | 37 | 73.125 |
| 15 | 14 | 78.730 | 39 | 38 | 89.535 |
| 16 | 15 | 65.940 | 40 | 39 | 78.385 |
| 17 | 16 | 40.210 | 41 | 40 | 89.535 |
| 18 | 17 | 63.795 | 42 | 41 | 82.405 |
| 19 | 18 | 75.955 | 43 | 42 | 89.490 |
| 20 | 19 | 73.465 | 44 | 43 | 82.170 |
| 21 | 20 | 79.175 | 45 | 44 | 88.455 |
| 22 | 21 | 74.435 | 46 | 45 | 82.170 |
| 23 | 22 | 76.580 | 47 | 46 | 81.220 |
| 24 | 23 | 80.595 | 48 | 47 | 64.415 |

compounds was diverse among all fractions. The details about number of compounds present in different fractions is tabulated in Table 9. Maximum number of compounds were identified in 26$^{th}$ sub-fraction (62) while less number of compounds were present in 40$^{th}$ sub-fraction (45). Almost 51 compounds identified in 28$^{th}$ sub-fraction and 34$^{th}$ sub-fraction while 53 compounds were identified in 38$^{th}$ fraction. From the whole fraction 60:40 (Hexane: ethyl acetate) 52 compounds were identified. Presence of unknown compound was ranged from 8 to 18. The 26$^{th}$ sub-fraction contained maximum number of unknown compounds (18) while least number of unknown compounds were present in 40$^{th}$ sub-fraction. The number of compounds only with amino acid structure were higher in 40$^{th}$ sub-fraction while only one such compound was present in Fraction (60:40 hexane: ethyl acetate).

### *4.1.5.2 Comparison of compounds in different fractions.*

Data obtained after LC-MS/MS was analysed and refined data is presented in Table 10. Among the compounds identified though LC-MS/MS analyses, eight compounds were present in all samples namely (24R)-1alpha,24,25,26-tetrahydroxyvitamin D2 / (24R)-1alpha,24,25,26-tetrahydroxyergocalciferol, 11alpha-hemiglutaryloxy-1,25- dihydroxy -vitamin D3, 18-Hydroxycortisol, cucurbitacin D, cucurbitacin I, cucurbitacin L, probucol spiroquuinone and saquinavir. Three compounds were found to present in five sub-fractions however absent in one sub-fraction *viz.* 18-hydroxycorticosterone (Absent in 34$^{th}$ sub-fraction), indoprofen (Absent in 28$^{th}$ sub-fraction) and norpropoxyphene (26$^{th}$ sub-fraction).

### *4.1.5.3 Frequency distribution of compounds in different curry leaf fractions*

The frequency distribution of different compounds in different sub-fractions are presented in Table 11-16. As per frequency distribution, for whole fraction (60:40 hexane: ethyl acetate) the maximum number of compounds (73.07%) were with peak area ranging from 11477760 to 5756137. In 26$^{th}$ sub-fraction peak area for maximum compounds (43.54%) were unknown. For 28$^{th}$ sub-fraction, maximum compounds (64.70%) were with peak area ranging from 7854246 to 3981987. In 34$^{th}$ sub-fraction, maximum compounds (66.66%) were with peak area ranging from 5347165.8 to 2737972.4. Majority of compounds (55.10%) in 38$^{th}$ sub-fraction were with peak area ranging from 8589818.4 to 4371156.2 while in 40$^{th}$ sub-fraction most of the compounds (62.22%) were with peak area ranging from 6573810 to 13114124.

**Table 9: Number of compounds present in different sub-fractions with high antioxidant activity in curry leaf**

| Sl. No. | Fraction | Compounds (No.) | | | |
|---|---|---|---|---|---|
| | | Known | Unknown | Peptides | Total |
| 1. | 60:40 (whole) | 38 | 13 | 1 | 52 |
| 2. | 26$^{th}$ sub-fraction | 41 | 18 | 3 | 62 |
| 3. | 28$^{th}$ sub-fraction | 34 | 14 | 3 | 51 |
| 4. | 34$^{th}$ sub-fraction | 34 | 12 | 5 | 51 |
| 5. | 38$^{th}$ sub-fraction | 36 | 10 | 3 | 49 |
| 6. | 40$^{th}$ sub-fraction | 31 | 8 | 6 | 45 |

**Table 10: Details of phytocompounds identified by LC-MS/MS from different fraction of curry leaf**

| Sl. No. | Name | Sample 1 | | Sample 2 | | Sample 3 | | Sample 4 | | Sample 5 | | Sample 6 | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | No. of times repeated (A) | Abundance (B) | A | B | A | B | A | B | A | B | A | B |
| 1 | (20R)-1alpha,25-dihydroxy-20-phenyl-16,17-didehydro-21-norvitamin D3 / (20R)-1alpha,25-dihydroxy-20- | | | | | | | | | | | 1 | 594358 |
| 2 | (24R)-1alpha,24,25,26-tetrahydroxyvitamin D2 / (24R)-1alpha,24,25,26-tetrahydroxyergocalciferol | 4 | 5210233 | 4 | 4341983 | 2 | 2862885 | 2 | 855737 | 2 | 678929 | 1 | 708377 |
| 3 | 1,2-dihexadecanoyl-3-(9Z-octadecenoyl)-sn-glycerol | | | | | | | 1 | 317520 | | | | |
| 4 | 11alpha-Hemiglutaryloxy-1,25-dihydroxyvitamin D3 | 4 | 1776069 | 2 | 1001396 | 2 | 1115840 | 3 | 1377754 | 4 | 1368502 | 4 | 1622179 |
| 5 | 17-Epistanozolol | | | 1 | 642095 | | | 1 | 643423 | 1 | 212701 | | |
| 6 | 18-Hydroxycorticosterone | 2 | 5237440 | 3 | 2834845 | 2 | 1489247 | | | 1 | 300099 | 1 | 2397529 |
| 7 | 18-Hydroxycortisol | 5 | 1961924 | 8 | 8083823 | 9 | | 7 | 8157874 | 9 | 7089903 | 9 | 11735166 |
| 8 | 1alpha,25-dihydroxy-22-oxavitamin D3 3-hemiglutarate/ 1alpha,25-dihydroxy-22-oxacholecalciferol 3-he | | | 1 | 216068 | | | | | | | | |
| 9 | 2,3-Diketo-13,14-dihydro-PGF1a | | | | | 1 | 883805 | | | | | | |
| 10 | 20beta-Dihydroprednisolone | | | 2 | 2738414 | 2 | 8606212 | 2 | 1889180 | 1 | 1944834 | | |

**Table 10 continued**

| Sl. No. | Name | Sample 1 | | Sample 2 | | Sample 3 | | Sample 4 | | Sample 5 | | Sample 6 | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | No. of times repeated (A) | Abundance (B) | A | B | A | B | A | B | A | B | A | B |
| 11 | 24-Nor-9,11-seco-11-acetoxy-3,6-dihydroxycholest-7,22-dien-9-one | | | 1 | 211001 | | | 1 | 158464 | | | | |
| 12 | 26,27-diethyl-1alpha,25-dihydroxy-22-thiavitamin D3 / 26,27-diethyl-1alpha,25-dihydroxy-22-thiachole | | | 1 | | | | | | | | | |
| 13 | 2-tetracosanamidoethanesulfoni | | 1 | 1 | 437299 | 1 | 401484 | 1 | 793972 | | | | |
| 14 | 3-Pyrrolidineacetic acid, 5-oxo-4,4-diphenyl- (9CI) | 3 | 481544 | 5 | 848112 | 4 | 1100986 | 2 | 1001123 | 2 | | | |
| 15 | 4-[[4-(4-fluorophenyl)-3-piperidinyl]methoxy]-2-methoxy-, (3S-trans)-Phenol | | | | | 1 | | 2 | | 1 | | | |
| 16 | 50xo-ETE-d7 | 1 | 5499407 | 1 | 4865090 | 1 | 5331780 | 1 | 480575 | | | | |
| 17 | 5-Methyltetrahydropteroyltri-L-glutamate | | | | | | | | | | | 2 | |
| 18 | 6beta-Hydroxyprednisolone | | | | | | | | | | | 1 | |
| 19 | alpha-Aminodiphenylacetic acid | | | 1 | 498869 | | | | | | | | |
| 20 | Ansamitocin P3 | | | | | | | 1 | | | | | |
| 21 | Arg Gly Met | 1 | 1244266 | | | | | | | | | | |
| 22 | Arg Thr Cys | | | | | | | | | | | 2 | 1226752 |
| 23 | Asn Ser Arg | | | | | 1 | | 1 | | 1 | | 1 | |

**Table 10 continued**

| Sl. No. | Name | Sample 1 | | Sample 2 | | Sample 3 | | Sample 4 | | Sample 5 | | Sample 6 | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | No. of times repeated (A) | Abundance (B) | A | B | A | B | A | B | A | B | A | B |
| 24 | Bilirubin | | | | | 1 | 227689 | | | | | | |
| 25 | Brassinolide | 1 | 246491 | 1 | 244252 | | | | | | | | |
| 26 | Cinnarizine | | | | | | | | | 1 | | 2 | 956828 |
| 27 | Cucurbitacin D | 1 | 606562 | 1 | 1843502 | 1 | 2271954 | 1 | 595723 | 1 | | 2 | 1241332 |
| 28 | Cucurbitacin I | 1 | 1681189 | 5 | 5229320 | 2 | 1596867 | 3 | 1695425 | 2 | | 2 | 1280358 |
| 29 | Cucurbitacin L | 3 | 1284199 | 3 | 1163034 | 4 | 1947614 | 3 | 1133231 | 4 | 1453151 | 2 | 969589 |
| 30 | Cys Lys Phe | | | | | 2 | 2228408 | | | | | | |
| 31 | Cys Tyr Cys | 2 | | | | | | | | | | | |
| 32 | Demethylcitalopram | | | 2 | | 1 | | 1 | | | | | |
| 33 | Dextromoramide M1 | | | | | | | | | 1 | | | |
| 35 | Dihydrodeoxystreptomycin | | | 1 | | | | 1 | | | | | |
| 36 | DL-2-Aminooctanoic acid | 1 | 983128 | | | | | | | 1 | | | |
| 37 | Dopexamine | | | 2 | 2813674 | 3 | 2602007 | 4 | 2796170 | 2 | 1078067 | | |
| 38 | Doxylamine | | | | | 1 | 1243977 | 1 | 1216484 | | | | |
| 39 | D-Phosphatidylglycerol | 2 | | 1 | | | | | | 1 | | | |
| 40 | Droperidol | 2 | 404580 | | | | | | | | | | |
| 41 | Erythromycin C | 2 | 229417 | 1 | | 2 | 1438666 | | | | | | |
| 42 | Fluoxetine | 1 | | | | | | | | | | | |
| 43 | Gibberellin A19 | 1 | 380398 | | | 1 | 1090108 | | | 1 | 7710620 | | |
| 44 | Gibberellin A36 | | | | | 4 | 4775238 | 8 | 13174750 | 4 | 3464655 | 7 | 14843617 |
| 45 | Gibberellin A44 diacid | 2 | 1475357 | | | | | | | 1 | 470063 | | |
| 46 | Gibberellin A53 | | | 1 | | | | | | | | | |
| 47 | GPEtn(12:0/0:0) | | | 1 | | | | | | | | | |

**Table 10 continued**

| Sl. No. | Name | Sample 1 | | Sample 2 | | Sample 3 | | Sample 4 | | Sample 5 | | Sample 6 | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | No. of times repeated (A) | Abundance (B) | A | B | A | B | A | B | A | B | A | B |
| 48 | GPEtn(20:0/18:1(13Z)) | 1 | | 1 | | | | | | | | | |
| 49 | Harderoporphyrin | | | 2 | | 2 | 1288140 | 3 | 693146 | 3 | 1820337 | | |
| 50 | His Ala Asp | | | 2 | | | | | | | | | |
| 51 | Histidinol | | | | | | | 1 | 623989 | | | 1 | 1133634 |
| 52 | Hydroxysaquinavir M2 | | | 1 | | | | | | | | | |
| 53 | Hydroxysaquinavir M3 | | | | | | | | | | | 2 | 413417 |
| 54 | Hydroxysaquinavir M7 | | | | | | | | | 1 | 349258 | | |
| 55 | Hydroxytinidazole glucuronide | 1 | 34513 | | | | | | | | | | |
| 56 | Ile Cys Leu | | | | | | | 1 | 11231462 | | | 1 | 11744369 |
| 57 | INDOPROFEN | 12 | 540970 | 4 | | | | 13 | 774828 | 14 | 2263103 | 11 | |
| 58 | Ipecac (Emetamine) | | | | | 1 | 195344 | | | | | | |
| 59 | KHAYANTHONE | | | | | 2 | | | | 1 | 318071 | 1 | 1387873 |
| 60 | Leu Lys Met | | | 1 | | | | | | | | | |
| 61 | Leu Met Lys | | | | | 1 | 1382848 | 1 | 738982 | 2 | 798302 | | |
| 62 | Linolenoyl lysolecithin | | | 1 | | | | | | | | | |
| 63 | Lorazepam | | | | | 1 | | | | | | | |
| 64 | LTB4-d4 | 1 | 599300 | | | | | 1 | 523957 | | | | |
| 65 | Lys Cys Asn | | | 1 | | | | | | | | | |
| 66 | Lys Met Ile | | | | | | | | | | | 3 | 19909131 |
| 67 | Lys Met Pro | | | | | | | 1 | 1271703 | 2 | 1040251 | | |
| 68 | Mefenamic acid Metabolite (Anthranilic acid, N-(a3-hydroxy-2,3-xylyl)-) | 3 | | 3 | | 4 | | 2 | | 1 | | 1 | |

**Continued Table 10**

| Sl. No. | Name | Sample 1 | | Sample 2 | | Sample 3 | | Sample 4 | | Sample 5 | | Sample 6 | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | No. of times repeated (A) | Abundance (B) | A | B | A | B | A | B | A | B | A | B |
| 69 | Met Met Met | | | 1 | | | | | | | | | |
| 70 | Met Pro Lys | | | | | | | 1 | 749924 | | | | |
| 71 | Met Val | | | 1 | | | | | | | | | |
| 72 | Monensin | 1 | 357753 | 1 | | 1 | 496911 | 1 | 270027 | | | | |
| 73 | Moricizine sulfoxide | 2 | | | | | | | | | | | |
| 74 | Morphinone | 2 | 2365234 | | | | | | | | | | |
| 75 | N-Desmethyltolmetin | | | | | | | | | 1 | | | |
| 76 | Norprochlorperazine | | | 5 | 11827543 | | | | | | | | |
| 77 | Norpropoxyphene | 7 | 28642632 | | | 3 | 19471025 | 2 | 7947739 | 4 | 21245805 | 2 | 10780464 |
| 78 | Octocrylene | | | 2 | 236228 | 1 | | | | | | | |
| 79 | Ouabain | | | | | | | 2 | | 2 | 299881 | | |
| 80 | Oxybutynin | 1 | 411000 | | | 2 | 1298614 | | | | | | |
| 81 | Phe Lys Cys | | | | | | | | | | | 2 | 3501443 |
| 82 | Pheniramine | | | | | | | 2 | 1931646 | 1 | 1243744 | | |
| 83 | Phorbol | | | | | | | | | | | 8 | 32735071 |
| 84 | PIPENZOLATE | 1 | | | | | | | | | | | |
| 85 | Probucol | | | 2 | 472836 | | | | | | | | |
| 86 | Probucol spiroquinone | 3 | 2096897 | 2 | 3578062 | 3 | 6907435 | 1 | 3802855 | 1 | | 1 | |
| 87 | PROMETON | | | | | | | 1 | 634757 | | | 1 | 1471037 |
| 88 | Propafenone | 1 | 493661 | | | 1 | 1311151 | | | | | 1 | 657937 |
| 89 | Protoporphyrinogen IX | | | | | | | | | | | 1 | 33495 |

**Table 10 continued**

| Sl. No. | Name | Sample 1 | | Sample 2 | | Sample 3 | | Sample 4 | | Sample 5 | | Sample 6 | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | No. of times repeated (A) | Abundance (B) | A | B | A | B | A | B | A | B | A | B |
| 90 | Retinyl glucuronide | | | | | | | | | 1 | | 2 | 1484979 |
| 91 | Saquinavir | 6 | 3158833 | 3 | 1889695 | 4 | 2930520 | 6 | 5650802 | 7 | 4841070 | 7 | 5403222 |
| 92 | Ser Gln Ile | | | | | | | | | | | 1 | 7372396 |
| 93 | Tetrahydrotrimethylhis pidin | 3 | 3770404 | 1 | | 1 | | 2 | | 2 | | 2 | |
| 94 | Tetranor-PGEM | 1 | 1351026 | | | | | | | 1 | 798938 | 1 | 1867972 |
| 95 | Trp Gln Lys | | | | | | | | | | | | |
| 96 | Val Val Phe | 1 | 4279627 | | | | | | | | | 1 | 580562 |
| 97 | Vitamin D3 glucosiduronate | | | 1 | 312506 | 1 | 271635 | 1 | 272335 | | | | |

**Table 11: Frequency distribution of compounds in fraction (60: 40) (Total compounds = 52)**

| Sl. No. | Peak area | Compound | |
|---|---|---|---|
| | | Number | Frequency (%) |
| 1 | 34364256-28642632- | 1 | 1.92 |
| 2 | 28642631-22921008 | 0 | 0 |
| 3 | 22921007-17199384 | 0 | 0 |
| 4 | 17199383-11477761 | 0 | 0 |
| 5 | 11477760-5756137 | 38 | 73.07 |
| 6 | 5756136-34513 | 1 | 1.92 |
| 7 | Undefined | 12 | 23.09 |

**Table 12: Frequency distribution of compounds in 26$^{th}$ sub-fraction (Total compounds = 62)**

| Sl. No. | Peak area | Compound | |
|---|---|---|---|
| | | Number | Frequency (%) |
| 1 | 14160627-11827543 | 1 | 1.61 |
| 2 | 11827542-9494459 | 1 | 1.61 |
| 3 | 9494458-7161376 | 1 | 1.61 |
| 4 | 7161375-4828292 | 6 | 9.67 |
| 5 | 4828291-2495209 | 25 | 40.32 |
| 6 | 2495208-162125 | 1 | 1.61 |
| 7 | Undefined | 27 | 43.54 |

**Table 13: Frequency distribution of compounds in 28th sub-fraction (Total compounds = 51)**

| Sl. No. | Peak area | Compound | |
|---|---|---|---|
| | | Number | Frequency (%) |
| 1 | 23343284-19471025 | 1 | 1.96 |
| 2 | 19471025-15598766 | 0 | 0 |
| 3 | 15598766-11726506 | 1 | 1.96 |
| 4 | 11726505-7854247 | 3 | 5.88 |
| 5 | 7854246-3981987 | 33 | 64.70 |
| 6 | 3981986-109728 | 1 | 1.96 |
| 7 | Undefined | 12 | 23.54 |

**Table 14: Frequency distribution of compounds in 34th sub-fraction (Total compounds = 51)**

| Sl. No. | Peak area | Compound | |
|---|---|---|---|
| | | Number | Frequency (%) |
| 1 | 15783944-13174750 | 2 | 3.92 |
| 2 | 13174749-10565555.6 | 1 | 1.96 |
| 3 | 10565554.6-7956361.2 | 2 | 3.92 |
| 4 | 7956360.2-5347166.8 | 2 | 3.92 |
| 5 | 5347165.8-2737972.4 | 34 | 66.66 |
| 6 | 2737971.4-128778 | 1 | 1.96 |
| 7 | Undefined | 9 | 17.66 |

**Table 15: Frequency distribution of compounds in 38th sub-fraction (Total compounds = 49)**

| Sl. No. | Peak area | Compound | |
|---|---|---|---|
| | | Number | Frequency (%) |
| 1 | 25464467-21245805 | 1 | 2.04 |
| 2 | 21245804-17027142.8 | 0 | 0 |
| 3 | 17027141.8-12808480.6 | 0 | 0 |
| 4 | 12808479.6-8589818.4 | 3 | 6.12 |
| 5 | 8589818.4-4371156.2 | 27 | 55.10 |
| 6 | 4371155.2-152494 | 1 | 2.04 |
| 7 | Undefined | 17 | 34.7 |

**Table 16: Frequency distribution of compounds in 40th sub-fraction (Total compounds = 45)**

| Sl. No. | Peak area | Compound | |
|---|---|---|---|
| | | Number | Frequency (%) |
| 1 | 39275386-32735971 | 1 | 2.22 |
| 2 | 32735071-26194756 | 1 | 2.22 |
| 3 | 26194755-19654441 | 1 | 2.22 |
| 4 | 19654440-13114125 | 4 | 8.88 |
| 5 | 13114124-6573810 | 28 | 62.22 |
| 6 | 33495-6573809 | 1 | 2.22 |
| 7 | Undefined | 9 | 20.02 |

#### *4.1.5.4 Abundant compounds in samples*

Details regarding ten most abundant compounds are presented in Table 17. norpropoxyphene was found to be abundant in whole fraction 60:40 (hexane: ethyl acetate), 28$^{th}$ sub-fraction and 38$^{th}$ fraction with peak area of 28642632, 19471025 and 21245805 respectively. In 26$^{th}$ sub-fraction, norprochlorperazine was most abundant with 11827543 peak area. The Gibberellin A36 was the most abundant compound in 34$^{th}$ sub-fraction (13174750). In case of 40$^{th}$ sub-fraction, compound phorbol was identified as most abundant with peak area of 32735071 identified. Phorbol recorded highest peak area (32735071) compared to all curry leaf compounds identified through LC-MS/MS in all fractions.

#### *4.1.5.4 Unique compounds in samples*

Some compounds were unique and was present in only one sub-fraction (Table 18). The 26$^{th}$ sub-fraction had the maximum number of unique compounds (13). It included morphinone, alpha-aminodiphenylacetic acid, probucol, 1alpha,25-dihydroxy-22- oxavitamin D3 3- hemiglutarate/ 1alpha,25- dihydroxy-22- oxacholecalciferol 3-he, GPETn(12:0/0:0), His Ala Asp, hydroxysaquinavir M2 ,Leu Lys Met, linolenoyl lysolecithin, Lys Cys Asn, Met Met Met, valylmethonine and 26,27-diethyl-1alpha,25-dihydroxy-22-thiavitamin D3 / 26,27-diethyl-1alpha,25- dihydroxy-22-thiachole. Among unique compound in 26$^{th}$ sub-fraction morphinone had highest peak area (23655234). The number of unique compounds varied among remaining sample of which 40$^{th}$ sub-fraction had ten unique compounds followed by six in whole fraction 60:40, five in 28$^{th}$ sub-fraction, two in 34$^{th}$ sub-fraction and in 38$^{th}$ sub-fraction.

### 4.2 IDENTIFICATION OF BIOACTIVE COMPOUNDS BY *IN SILICO* ANALYSIS

Seventy-nine ligands were identified from curry leaf through LC-MS/MS analysis and 19 approved drugs were selected for molecular docking. Structure of ligands and approved drugs were drawn or retrieved from database like PubChem and Chemspider. The details of chemical properties like chemical formula and molecular weight of ligands is presented in Table 19 and 20. Total 23 targets were selected for molecular docking. Selection was based on literature survey and from Potential Drug Target Database. For cancer, eight target proteins were selected while for diabetes seven targets were selected, four targets were selected each for Arthritis and Alzheimer's. Details of targets are presented in Table 20.

**Table 17: Most abundant compounds in different sub-fractions with high antioxidant activity in curry leaf**

| Sl. No. | Fraction | Compound | | |
|---|---|---|---|---|
| | | Sl. No. | Name | Abundance (Peak) |
| 1 | 60:40 (whole) | 1 | Norpropoxyphene | 28642632 |
| | | 2 | 50oxo-ETE-d7 | 5499407 |
| | | 3 | 18-Hydroxycorticosterone | 5237440 |
| | | 4 | (24R)-1alpha,24,25,26-tetrahydroxyvitamin D2 / (24R)-1alpha,24,25,26-tetrahydroxyergocalciferol | 5210233 |
| | | 5 | Val Val Phe | 4279627 |
| | | 6 | Tetrahydrotrimethylhis Pidin | 3770404 |
| | | 7 | Saquinavir | 3158833 |
| | | 8 | Morphinone | 2365234 |
| | | 9 | Probucol spiroquinone | 2096897 |
| | | 10 | 18-Hydroxycortisol | 1961924 |
| 2 | 26th | 1 | Norprochlorperazine | 11827543 |
| | | 2 | 18-Hydroxycortisol | 8083823 |
| | | 3 | 50oxo-ETE-d7 | 4865090 |
| | | 4 | (24R)-1alpha,24,25,26-tetrahydroxyvitamin D2 / (24R)-1alpha,24,25,26-tetrahydroxyergocalciferol | 4341983 |
| | | 5 | Cucurbitacin I | 3647712 |
| | | 6 | Probucol spiroquinone | 3578062 |
| | | 7 | 18-Hydroxycorticosterone | 2834845 |
| | | 8 | Dopexamine | 2813674 |
| | | 9 | 20beta-Dihydroprednisolone | 2738414 |
| | | 10 | Saquinavir | 1889695 |
| 3 | 28th | 1 | Norpropoxyphene | 19471025 |
| | | 2 | 20beta-Dihydroprednisolone | 8606212 |
| | | 3 | Probucol spiroquinone | 6907435 |
| | | 4 | 50oxo-ETE-d7 | 5331780 |
| | | 5 | Gibberellin A36 | 4775238 |
| | | 6 | Saquinavir | 2930520 |
| | | 7 | (24R)-1alpha,24,25,26-tetrahydroxyvitamin D2 / (24R)-1alpha,24,25,26-tetrahydroxyergocalciferol | 2862885 |
| | | 8 | Dopexamine | 2602007 |
| | | 9 | Cucurbitacin D | 2271954 |
| | | 10 | Cys Lys Phe | 2228408 |

**Table 17 Continued**

| Sl. No. | Fraction | Compound | | |
|---|---|---|---|---|
| | | Sl. No. | Name | Abundance (Peak) |
| 4 | 34th | 1 | Gibberellin A36 | 13174750 |
| | | 2 | Ile Cys Leu | 11231462 |
| | | 3 | 18-Hydroxycortisol | 8157874 |
| | | 4 | Norpropoxyphene | 7947739 |
| | | 5 | Saquinavir | 5650802 |
| | | 6 | Probucol spiroquinone | 3802855 |
| | | 7 | Dopexamine | 2796170 |
| | | 8 | Pheniramine | 1931646 |
| | | 9 | 20beta-Dihydroprednisolone | 1889180 |
| | | 10 | Cucurbitacin I | 1695425 |
| 5 | 38th | 1 | Norpropoxyphene | 21245805 |
| | | 2 | Gibberellin A19 | 7710620 |
| | | 3 | 18-Hydroxycortisol | 7089903 |
| | | 4 | Saquinavir | 4841070 |
| | | 5 | Gibberellin A36 | 3464655 |
| | | 6 | INDOPROFEN | 2263103 |
| | | 7 | 20beta-Dihydroprednisolone | 1944834 |
| | | 8 | 16.368 | 1495782 |
| | | 9 | Cucurbitacin L | 1453151 |
| | | 10 | 11alpha-Hemiglutaryloxy-1,25-dihydroxyvitamin D3 | 1368502 |
| 6 | 40th | 1 | Phorbol | 32735071 |
| | | 2 | Lys Met Ile | 19909131 |
| | | 3 | Gibberellin A36 | 14843617 |
| | | 4 | Ile Cys Leu | 11744369 |
| | | 5 | 18-Hydroxycortisol | 11735166 |
| | | 6 | Norpropoxyphene | 10780464 |
| | | 7 | Ser Gln Ile | 7372396 |
| | | 8 | Saquinavir | 5403222 |
| | | 9 | Phe Lys Cys | 3501443 |
| | | 10 | 18-Hydroxycorticosterone | 2397529 |

**Table 18: Unique compounds in different sub-fractions with high antioxidant activity in curry leaf**

| Sl. No. | Fraction | Compounds | | |
|---|---|---|---|---|
| | | Name | | Abundance |
| 1 | 60:40 (whole) | 1 | Arg Gly Met | 1244266 |
| | | 2 | Droperidol | 404580 |
| | | 3 | Hydroxytinidazole glucuronide | 34513 |
| | | 4 | Fluoxetine | - |
| | | 5 | Moricizine sulfoxide | - |
| | | 6 | Cys Tyr Cys | - |
| 2 | 26th | 1 | Morphinone | 2365234 |
| | | 2 | Alpha-Aminodiphenylacetic acid | 498869 |
| | | 3 | Probucol | 472836 |
| | | 4 | 1alpha,25-dihydroxy-22- oxavitamin D3 3-hemiglutarate/ 1alpha,25- dihydroxy-22-oxacholecalciferol 3-he | 216068 |
| | | 5 | GPETn(12:0/0:0) | - |
| | | 6 | His Ala Asp | - |
| | | 7 | Hydroxysaquinavir M2 | - |
| | | 8 | Leu Lys Met | - |
| | | 9 | Linolenoyl lysolecithin | - |
| | | 10 | Lys Cys Asn | - |
| | | 11 | Met Met Met | - |
| | | 12 | Valylmethionine | - |
| | | 13 | 26,27-diethyl-1alpha,25- dihydroxy-22-thiavitamin D3 / 26,27-diethyl-1alpha,25-dihydroxy-22-thiachole | - |
| 3 | 28th | 1 | Cys Lys Phe | 2228408 |
| | | 2 | 2,3-Diketo-13,14-dihydro- PGF1a | 883805 |
| | | 3 | Bilirubin | 227689 |
| | | 4 | Ipecac (Emetamine) | 195344 |
| | | 5 | Lorezepam | - |
| 4 | 34th | 1 | 1,2-dihexadecanoyl-3-(9Z- octadecenoyl)-sn-glycerol | 317520 |
| | | 2 | Ansamitocin P3 | - |

**Table 18 Continued**

| Sl. No. | Fraction | Compounds | | |
|---|---|---|---|---|
| | | Name | | Abundance |
| 5 | 38th | 1 | Hydroxysaquinavir M7 | 349258 |
| | | 2 | Dextromoramide M1 | - |
| 6 | 40th | 1 | Phorbol | 32735071 |
| | | 2 | Ser Gln Ile | 7372396 |
| | | 3 | Phe Lys Cys | 3501443 |
| | | 4 | Arg Thr Cys | 1226752 |
| | | 5 | (20R)-1alpha,25-dihydroxy-20- phenyl-16,17-didehydro-21- norvitamin D3 / (20R)- 1alpha,25-dihydroxy-20- | 594358 |
| | | 6 | Hydroxysaquinavir M3 | 413417 |
| | | 7 | Protoporphyrinogen IX | 33495 |
| | | 8 | 6beta-Hydroxyprednisolone | - |
| | | 9 | DIHYDRO-7-DESACETYLDEOXYGEDUNIN | - |
| | | 10 | 5-Methyltetrahydropteroyltri-L- glutamate | - |

**Table 19: Chemical properties of ligands from curry leaf**

| Sl. No. | Compound Name | Source ID* | Chemical Formula | Molecular Weight (g/mol) |
|---|---|---|---|---|
| 1 | 4-[[4-(4-fluorophenyl)-3-piperidinyl]methoxy]-2- methoxy, (3S-trans)-Phenol | 46781621 | $C_{19}H_{22}FNO_3$ | 331.1571 |
| 2 | 6 beta-Hydroprednisolone | 14726612 | $C_{21}H_{28}O_6$ | 376.1915 |
| 3 | 18-Hydroxycortisol | 44263343 | $C_{21}H_{30}O_6$ | 378.2063 |
| 4 | 18-Hydroxycortisterone | 11222 | $C_{21}H_{30}O_5$ | 362.2108 |
| 5 | 20 Beta Hydroxypredanisolone | 13962390 | $C_{21}H_{30}O_5$ | 362.2117 |
| 6 | Alpa-Aminodiphenylacetic Acid | 182869 | $C_{14}H_{13}NO_2$ | 227.0945 |
| 7 | Anasamitocin P3 | 5282049 | $C_{32}H_{43}ClN_2O_9$ | 634.2666 |
| 8 | Bilirubin | 5282049 | $C_{33}H_{36}N4O_6$ | 584.2668 |
| 9 | Brassinolide | 115196 | $C_{28}H_{48}O_6$ | 480.3486 |
| 10 | Cinnarizine | 1547484 | $C_{26}H_{28}N_2$ | 368.2251 |
| 11 | Cucurbitacin D | 5281318 | $C_{30}H_{44}O_7$ | 514.2993 |
| 12 | Cucurbitacin I | 5281321 | $C_{30}H_{42}O_7$ | 514.299 |
| 13 | Cucurbitacin L | 441820 | $C_{30}H_{44}O_7$ | 516.3099 |
| 14 | Demethylcitalopram | 162180 | $C_{19}H_{19}FN_2O$ | 310.1445 |
| 15 | Dihydro-7-Desacetyldeoxygedunin | | $C_{26}H_{34}O_5$ | 426.2429 |
| 16 | Dihydrodeoxystreptomycin | 11953824 | $C_{21}H_{41}N_7O_{11}$ | 567.2882 |
| 17 | DL-2-Aminooctanoic Acid | 69522 | $C_8H_{17}NO_2$ | 159.125 |
| 18 | Dopexamine | 55483 | $C_{22}H_{32}N_2O_2$ | 356.2459 |
| 19 | Doxylamine | 3162 | $C_{17}H_{22}N_2O$ | 270.1728 |
| 20 | Droperidol | 3168 | $C_{22}H_{22}FN_3O_2$ | 379.1672 |
| 21 | Emetamine | 442217 | $C_{29}H_{36}N_2O_4$ | 476.271 |
| 22 | Epistanazolol | 6429888 | $C_{21}H_{32}N_2O$ | 328.251 |
| 23 | Erythromycin C | 83933 | $C_{36}H_{65}NO_{13}$ | 719.4437 |
| 24 | Flucoxetine | 3386 | $C_{17}H_{18}F_3NO$ | 309.1356 |
| 25 | Gibberlin A 36 | | $C_{20}H_{26}O_6$ | 362.1758 |
| 26 | Gibberlin A44 Diacid | | $C_{20}H_{28}O_6$ | 364.1898 |
| 27 | Gibberlin A19 | | $C_{20}H_{26}O_6$ | 362.1757 |
| 28 | Harderoporphyrin | 3081462 | $C_{35}H_{36}N_4O_6$ | 608.2636 |
| 29 | Histidinol | 776 | $C_6H_{11}N_3O$ | 141.0895 |
| 30 | Indoprofen | 3718 | $C_{17}H_{15}NO_3$ | 281.1049 |
| 31 | Khayanthone | 6708528 | $C_{32}H_{42}O_9$ | 570.2864 |
| 32 | Lorazepam | 3958 | $C_{15}H_{10}Cl_2N_2O_2$ | 320.0163 |
| 33 | Monensin | 441145 | $C_{36}H_{62}O_{11}$ | 670.4335 |
| 34 | Moricizine Sulfoxide | 6454358 | $C_{22}H_{25}N_3O_5S$ | 443.1434 |
| 35 | Morphionone | 5459823 | $C_{17}H_{17}NO_3$ | 283.12 |
| 36 | Norpochlorperazine | 10451115 | $C_{19}H_{22}ClN_3S$ | 359.1213 |
| 37 | Norpropoxyphene | 18804 | $C_{21}H_{27}NO_2$ | 325.2054 |
| 38 | Octocrylene | 22571 | $C_{24}H_{27}NO_2$ | 361.2039 |
| 39 | Ouabain | 439501 | $C_{29}H_{44}O_{12}$ | 584.2871 |
| 40 | Oxybutylnin | 4634 | $C_{22}H_{31}NO_3$ | 357.2307 |
| 41 | Pheniramine | 4761 | $C_{16}H_{20}N_2$ | 240.162 |

**Table 19 continued**

| Sl. No. | Compound Name | Source ID* | Chemical Formula | Molecular Weight (g/mol) |
|---|---|---|---|---|
| 42 | Phorbol | 4042070 | $C_{20}H_{28}O_6$ | 364.1915 |
| 43 | Probucol | 4912 | $C_{31}H_{48}O_2S_2$ | 516.3136 |
| 44 | Prometon | 4928 | $C_{10}H_{19}N_5O$ | 225.1603 |
| 45 | Propafenone | 4932 | $C_{21}H_{27}NO_3$ | 341.1997 |
| 46 | Protoporphyrinogen IX | 121893 | $C_{34}H_{40}N_4O_4$ | 568.3058 |
| 47 | Arg Thr Cys 16(6) | | $C_{13}H_{26}N_6O_5S$ | 378.1716 |
| 48 | Asn Ser Arg 59(5) | | $C_{13}H_{25}N_7O_6$ | 375.1849 |
| 49 | Cys Lys Phen 11(3) | | $C_{18}H_{28}N_4O_4S$ | 396.1818 |
| 50 | His Ala Asp 48(2) | | $C_{13}H_{19}N_5O_6$ | 341.1316 |
| 51 | Hydroxysquanavir M2 | | $C_{38}H_{50}N_6O_6$ | 686.3726 |
| 52 | Hydroxysquanavir M7 | | $C_{38}H_{50}N_6O_6$ | 686.3725 |
| 53 | Ile Cys Leu 50(4) | | $C_{15}H_{29}N_3O_4S$ | 347.1893 |
| 54 | Leu Lys Met 5 (2) | | $C_{17}H_{34}N_4O_4S$ | 390.23 |
| 55 | Leu Met Lys 20(3) | | $C_{17}H_{34}N_4O_4S$ | 390.2302 |
| 56 | Linolyl Lysothin | | $C_{26}H_{48}NO_7P$ | 517.3151 |
| 57 | Lys Met Ile 18(6) | | $C_{17}H_{34}N_4O_4S$ | 390.2307 |
| 58 | Lys Met Pro 13(4) | | $C_{16}H_{30}N_4O_4S$ | 374.199 |
| 59 | Met Met Met 64(2) | | $C_{15}H_{29}N_3O_4S3$ | 411.1346 |
| 60 | Met Pro Lys 12(4) | | $C_{16}H_{30}N_4O_4S$ | 374.199 |
| 61 | Phe Lys Cys6(6) | | $C_{18}H_{28}N_4O_4S$ | 396.1822 |
| 62 | Ser Gln Ile 54(6) | | $C_{14}H_{26}N_4O_6$ | 346.1824 |
| 63 | Dextroramide M1 | | $C_{21}H_{26}N_2O$ | 322.2043 |
| 64 | 2-Tetracpsanamidoethamesulfoni | | $C_{26}H_{53}NO_4S$ | 475.3678 |
| 65 | 3-Pyrrolidineacetic acid, 5-oxo-4,4-diphenyl- (9CI) | | $C_{18}H_{17}NO_3$ | 295.1198 |
| 66 | 50-Oxodt | | $C_{20}H_{23}D_7O_3$ | 4865090 |
| 67 | Cys Tyr Cys | | $C_{15}H_{21}N_3O_5S_2$ | 387.0953 |
| 68 | D-Phosphatidyl Glycerol | | $C_{32}H_{63}O_{12}P$ | 670.4028 |
| 69 | Gpetn | | $C_{43}H_{84}NO_8P$ | 773.59 |
| 70 | Hydroxysqaquinavir M3 | | $C_{38}H_{50}N_6O_6$ | 686.3724 |
| 71 | Hydroxytinidazole Glucuronide | | $C_{14}H_{21}N_3O_{11}S$ | 439.0922 |
| 72 | Ltb4-B4 | | $C_{20}H_{28}D_4O_4$ | 340.2523 |
| 73 | N Dismethyltolmetin | | $C_{14}H_{13}NO_3$ | 243.0896 |
| 74 | Probucol Squinone | | $C_{31}H_{46}O_2S_2$ | 514.2974 |
| 75 | Saquinavir | 6440956 | $C_{38}H_{50}N_6O_5$ | 670.3792 |
| 76 | Tetrahydritrimethylispidin | | $C_{16}H_{20}O_5$ | 292.1336 |
| 77 | Tetramer-PGEM | | $C_{16}H_{22}O_7$ | 326.1382 |
| 78 | Valylmethionine | 292427 | $C_{10}H_{20}N_2O_3S$ | 378.2061 |
| 79 | Vitamin D3 Glucosiduronate | 24779623 | $C_{33}H_{52}O_7$ | 560.3771 |

**Table 20: Chemical properties of ligands of commercial drugs**

| Sl. No. | Drug Name | Source ID* | Chemical Formula | Molecular Weight (g/mol) |
|---|---|---|---|---|
| 1 | Apigenin | 5280443 | $C_{15}H_{10}O_5$ | 270.24 |
| 2 | Dexamethasone | 5743 | $C_{22}H_{29}FO_5$ | 392.50 |
| 3 | Diclofenac | 3033 | $C_{14}H_{11}Cl_2NO_2$ | 296.10 |
| 4 | Dicloroacetci acid | 6597 | $C_2H_2Cl_2O_2$ | 128.94 |
| 5 | Diphenylamine | 11487 | $C_{12}H_{11}N$ | 169.22 |
| 6 | Epalrestat | 1549120 | $C_{15}H_{13}NO_3S_2$ | 319.40 |
| 7 | Fenretinide | 5288209 | $C_{26}H_{33}NO_2$ | 391.50 |
| 8 | Fulvestrant | 104741 | $C_{32}H_{47}F_5O_3S$ | 606.80 |
| 9 | Galanthamine | 9651 | $C_{17}H_{21}NO_3$ | 287.35 |
| 10 | Lithium carbonate | 11125 | $CLi_2O_3$ | 73.90 |
| 11 | Myricetin | 5281672 | $C_{15}H_{10}O_8$ | 318.23 |
| 12 | Nsc-54776 | 104758 | - | - |
| 13 | Regorafenib | 11167602 | $C_{21}H_{15}ClF_4N_4O_3$ | 482.80 |
| 14 | Ribociclib | 44631912 | $C_{23}H_{30}N_8O$ | 434.50 |
| 15 | Rivastigmine | 77991 | $C_{14}H_{22}N_2O_2$ | 250.34 |
| 16 | Tamoxifen | 2733526 | $C_{26}H_{29}NO$ | 371.50 |
| 17 | Technetium tc99m medronate | 131704315 | $CH_{60}P_2Tc$ | 305.91 |
| 18 | Trimetrexate | 5583 | $C_{19}H_{23}N_5O_3$ | 369.40 |
| 19 | Verubecestat | 51352361 | $C_{17}17_{10}F_{23}N_5O_3$ | 409.40 |

### 4.2.1 Retrieval of structure of ligands

Structure of 45 ligands were retrieved from PubChem and Chemspider database and were downloaded in 3D format. Structure for remaining ligands were drawn by using ChemSketch software and saved in 3D format. Structure for all approved drugs were retrieved from PubChem database and stored in 3D format.

Ligands were prepared by filtering them through Lipinski Veber rule of five in Discovery Studio 4.0. Result of Lipinski Veber rule of five presented in Table 21. Among 79 ligands of curry leaf 43 ligands were passed while remaining 32 failed to pass. Among 19 commercial approved drugs one drug Fulvestrant failed to pass Lipinski Veber rule while rest 18 passed (Table 22).

### 4.2.2 Retrieval of structure of targets

Different targets identified through literature survey and Potential Drug Target Database included 17 beta-HSD (Breast Cancer), polo-like kinase 1 (Breast Cancer), exchange protein directly activated by CAMP (Pancreatic cancer), Nat -2 receptor (All type of cancer), phosphoinotidate-3 kinase (All type of cancer), human androgen receptor (Prostate Cancer), dihydropholate reductase (Colon cancer) and human estrogen receptor ligand-binding domain (Breast Cancer) for cancer, fructose-1, 6-bisphosphatase, human glucokinase, glycogen synthase kinase, pyruvate dehydrogenase kinase, Human aldose reductase, human multidrug resistance protein and human dipeptidyl peptidase IV for diabetes. For arthritis, nitric oxide synthase, cyclooxygenase, vascular endothelial growth factor and glucocorticoid receptor were selected while for Alzheimer's human beta-secretase 1, tau protein kinase, human acetylcholinestarase and human butyrylcholinesterase and were selected. Structure retrieved from protein data bank are presented in Figures 3-6. Protein targets were prepared using prepare protein tool of Discovery Studio 4.

### 4.2.3 Prediction of active sites of the targets

Discovery Studio tools were used to predict active sites in target proteins. It was done with help of "Receptor cavity" and "Current selection" tools. Active sites of targets for various diseases are presented in Table 23. Highest number of active was present in NAT-2 receptor (34) followed by 27 for dipeptidyl peptidase-4, 24 for cyclooxygenase and 17 for nitrous oxide synthase while single active site was present in phosphoinositide -3 kianse, human beta-secretase and androgen receptor. The

**Table 21: Filtration of ligands from curry leaf using Lipinski and Veber rules**

| Sl. No. | Compound name | Source ID* | A log p value | No. of Rotatable bonds | Hydrogen bond acceptor No. | Hydrogen bond donor No. | Pass /fail |
|---|---|---|---|---|---|---|---|
| 1 | 4-[[4-(4-fluorophenyl)-3- piperidinyl]methoxy]-2-methoxy-, (3S-trans)-Phenol | 46781621 | 0.192 | 3 | 6 | 4 | Pass |
| 2 | 6beta-Hydroprednisolone | 14726612 | 0.158 | 2 | 6 | 4 | Pass |
| 3 | 18-hydroxycortisol | 44263343 | 0.192 | 3 | 6 | 4 | Pass |
| 4 | 18-hydroxycortisterone | 11222 | 0.928 | 3 | 5 | 3 | Pass |
| 5 | 20 beta hydroxypredanisolone | 13962390 | 1.086 | 2 | 5 | 4 | Pass |
| 6 | Alpa-Aminodiphenylacetic acid | 182869 | 0.625 | 3 | 3 | 1 | Pass |
| 7 | Anasamitocin P3 | 5282049 | 4.445 | 5 | 9 | 2 | Fail |
| 8 | Bilirubin | 5282049 | 2.107 | 4 | 6 | 12 | Fail |
| 9 | Brassinolide cid | 115196 | 3.572 | 5 | 6 | 4 | Pass |
| 10 | cinnarizine | 1547484 | 5.524 | 6 | 2 | 0 | Pass |
| 11 | Cucurbitacin D | 5281318 | 1.933 | 4 | 7 | 4 | Pass |
| 12 | Cucurbitacin I | 5281321 | 1.456 | 4 | 7 | 3 | Pass |
| 13 | Cucurbitacin L | 441820 | 1.345 | 5 | 7 | 3 | Pass |
| 14 | Demethylcitalopram | 162180 | 1.954 | 5 | 2 | 1 | Pass |
| 15 | Dihydro-7-desacetyldeoxygedun | 6708643 | 3.861 | 1 | 4 | 1 | Pass |
| 16 | Dihydrodeoxystreptomycin | 11953824 | -7.275 | 9 | 11 | 14 | Fail |

**Table 21 continued**

| Sl. No. | Compound name | Source ID* | A log p value | No. of Rotatable bonds | Hydrogen bond acceptor No. | Hydrogen bond donor No. | Pass /fail |
|---|---|---|---|---|---|---|---|
| 18 | Dopexamine | 55483 | 1.805 | 13 | 2 | 4 | Fail |
| 19 | Doxylamine | 3162 | 1.488 | 6 | 2 | 2 | Pass |
| 20 | Droperidol | 3168 | 2.44 | 6 | 3 | 2 | Pass |
| 21 | Emetamine | 442217 | 3.578 | 7 | 5 | 1 | Pass |
| 22 | Epistanazolol | 6429888 | 7.993 | 3 | 1 | 1 | Pass |
| 23 | Erythromycin C | 83933 | -0.177 | 6 | 13 | 7 | Fail |
| 24 | Flucoxetine_ | 3386 | 2.802 | 7 | 1 | 1 | Pass |
| 25 | Gibberlin A 36 | 443455 | 0.094 | 3 | 6 | 2 | Pass |
| 26 | Gibberlin A44 Diacid | 40915 | -0.142 | 3 | 6 | 3 | Pass |
| 27 | Gibberlin A19 | 5460209 | -1.616 | 3 | 6 | 2 | Pass |
| 28 | Harderoporphyrin | 3081462 | -2.459 | 10 | 8 | 3 | Pass |
| 29 | Histidinol | 776 | -1.23 | 3 | 1 | 4 | Pass |
| 30 | Indoprofen | 3718 | 1.227 | 3 | 3 | 0 | Pass |
| 31 | Khayanthone | 6708528 | 2.677 | 7 | 8 | 0 | Pass |
| 32 | Lorazepam | 3958 | 3.509 | 1 | 3 | 2 | Pass |
| 33 | Monensin | 441145 | 2.01 | 10 | 11 | 3 | Fail |
| 34 | Moricizine Sulfoxide | 6454358 | 0.366 | 6 | 6 | 1 | Pass |

**Table 21 continued**

| Sl. No. | Compound name | Source ID* | A log p value | No. of Rotatable bonds | Hydrogen bond acceptor No. | Hydrogen bond donor No. | Pass /fail |
|---|---|---|---|---|---|---|---|
| 35 | Morphionone | 5459823 | 0.131 | 0 | 3 | 2 | Pass |
| 36 | Norpochlorperazine | 10451115 | 1.257 | 4 | 2 | 2 | Pass |
| 37 | Norpropoxyphene | 18804 | 2.944 | 9 | 2 | 1 | Pass |
| 38 | Octocrylene | 22571 | 6.487 | 10 | 3 | 0 | Pass |
| 39 | Ouabain | 439501 | -2.172 | 4 | 12 | 8 | Fail |
| 40 | Oxybutylnin | 4634 | 3.085 | 10 | 3 | 2 | Pass |
| 41 | Pheniramine | 4761 | 1.471 | 5 | 2 | 1 | Pass |
| 42 | Phorbol | 402070 | -52 | 1 | 6 | 5 | Pass |
| 43 | Probucol | 4912 | 9.779 | 8 | 4 | 2 | Fail |
| 44 | Prometon | 4928 | 2.562 | 5 | 6 | 2 | Pass |
| 45 | Propafenone | 4932 | 2.442 | 11 | 3 | 2 | Fail |
| 46 | Protoporphyrinogen IX | 121893 | 5.26 | 8 | 4 | 4 | Fail |
| 47 | Arg Thr Cys 16(6) | | -4.833 | 12 | 11 | 11 | Fail |
| 48 | Asn Ser Arg59(5) | | -6.981 | 13 | 13 | 13 | Fail |
| 49 | Cys Lys Phen 11(3) | | -3.518 | 12 | 8 | 8 | Fail |
| 50 | His Ala Asp48(2) | | -4.428 | 9 | 9 | 6 | Pass |
| 51 | Hydroxysquanavir M2 | | 3.085 | 13 | 11 | 6 | Fail |

**Table 21 continued**

| Sl. No. | Compound name | Source ID* | A log p value | No. of Rotatable bonds | Hydrogen bond acceptor No. | Hydrogen bond donor No. | Pass /fail |
|---|---|---|---|---|---|---|---|
| 52 | Hydroxysquanavir M7 | | 2.197 | 14 | 12 | 7 | Fail |
| 53 | Ile Cys Leu 50(4) | | -1.459 | 10 | 7 | 5 | Pass |
| 54 | Leu Lys Met 5 (2) | | -1.054 | 14 | 7 | 5 | Fail |
| 55 | Leu Met Lys 20 (3) | | -5.224 | 13 | 12 | 8 | Fail |
| 56 | Linolyl Lysothin | | 3.479 | 23 | 8 | 4 | Fail |
| 57 | Lys Met Ile 18(6) | | 3.479 | 23 | 8 | 4 | Fail |
| 58 | Lys Met Pro 13(4) | | -4.512 | 11 | 8 | 7 | Fail |
| 59 | Met Met Met 64(2) | | -3.091 | 14 | 7 | 5 | Fail |
| 60 | Met Pro Lys 12(4) | | -4.512 | 11 | 8 | 7 | Fail |
| 61 | Phe Lys Cys 6(6) | | -3.518 | 12 | 8 | 8 | Fail |
| 62 | Ser Gln Ile 54(6) | | -4.588 | 11 | 10 | 8 | Fail |
| 63 | Dextroramidemi M1 | | 3.348 | 1 | 0 | 0 | Pass |
| 64 | 2-Tetracpsanamidoethamesulfoni | | 8.646 | 26 | 5 | 4 | Fail |
| 65 | Pyrrolidineacetic acid, 5-oxo- 4,4-diphenyl- (9CI) | | 2.092 | 4 | 3 | 1 | Pass |
| 66 | 50-Oxodt | | 8.646 | 26 | 4 | 5 | Fail |
| 67 | Cys Tyr Cys | | -3.27 | 6 | 7 | 8 | Fail |
| 68 | D-Phosphatidyl Glycerol | | 4.704 | 36 | 2 | 12 | Fail |

**Table 21 continued**

| Sl. No. | Compound name | Source ID* | A log p value | No. of Rotatable bonds | Hydrogen bond acceptor No. | Hydrogen bond donor No. | Pass /fail |
|---|---|---|---|---|---|---|---|
| 69 | Gpetn | | 12.75 | 43 | 3 | 7 | Fail |
| 70 | Hydroxysqaquinavir M3 | | 3.322 | 13 | 6 | 7 | Fail |
| 71 | Hydroxytinidazole Glucuronide | | 1.222 | 9 | 7 | 9 | Fail |
| 72 | Ltb4-B4 | | 0.422 | 17 | 6 | 8 | Fail |
| 73 | N Dismethyltolmetin | | 3.667 | 13 | 5 | 7 | Fail |
| 74 | Probucol Squinone | | 0.907 | 3 | 2 | 0 | Fail |
| 75 | Saquinavir | 6440956 | 2.223 | 8 | 7 | 3 | Pass |
| 76 | Tetrahydritrimethylispidin | | 2.495 | 6 | 5 | 0 | Pass |
| 77 | Tetramer-PGEM | | -1.02 | 10 | 6 | 0 | Pass |
| 78 | Valylmethionine | 292427 | 0.221 | 7 | 4 | 1 | Pass |
| 79 | Vitamin D3 Glucosiduronate | 24779623 | 4.662 | 9 | 7 | 3 | Pass |

**Table 22: Filtration of commercial approved drugs using Lipinski and Veber rules**

| Sl. No. | Compound name | Source ID* | A log p value | No. of Rotatable bonds | Hydrogen bond acceptor No. | Hydrogen bond donor No. | Pass /fail |
|---|---|---|---|---|---|---|---|
| 1 | Apigenin | 5280443 | 1.707 | 1 | 5 | 2 | Pass |
| 2 | Dexamethasone | 5743 | 1.708 | 2 | 5 | 3 | Pass |
| 3 | Diclofenac | 3033 | 2.899 | 4 | 3 | 1 | Pass |
| 4 | Dichloroacetic acid | 6597 | -0.484 | 1 | 2 | 0 | Pass |
| 5 | Diphenylamine | 11487 | 3.38 | 2 | 1 | 1 | Pass |
| 6 | Epalrestat | 1549120 | 1.968 | 4 | 5 | 0 | Pass |
| 7 | Fenretinide | 5288209 | 6.463 | 6 | 2 | 2 | Pass |
| 8 | Fulvestrant | 17756771 | 8.437 | 15 | 3 | 2 | Fail |
| 9 | Galanthamine | 9651 | 1.442 | 1 | 4 | 1 | Pass |
| 10 | Lithium carbonate | 11125 | -1.965 | 0 | 3 | 0 | Pass |
| 11 | Myricetin | 5281672 | -0.018 | 1 | 8 | 4 | Pass |
| 12 | Nsc-54776 | 104758 | -0.608 | 9 | 9 | 2 | Pass |
| 13 | Regorafenib | 11167602 | 4.381 | 6 | 4 | 3 | Pass |
| 14 | Ribociclib | 44631912 | 1.167 | 5 | 4 | 3 | Pass |
| 15 | Rivastigmine | 77991 | 1.038 | 5 | 2 | 1 | Pass |
| 16 | Tamoxifen | 2733526 | 4.758 | 8 | 1 | 1 | Pass |
| 17 | Technetium tc99m medronate | 131704315 | -4.313 | 2 | 6 | 2 | Pass |
| 18 | Trimetrexate | 5583 | 2.638 | 6 | 7 | 4 | Pass |
| 19 | Verubecestat | 51352361 | 1.21 | 3 | 8 | 4 | Pass |

**Table 23: Prediction of active sites of targets for different diseases**

| Sl. No. | Target protein | Total No. of active sites | Best active site | Critical amino acid at selected active site |
|---|---|---|---|---|
| 1 | 17-beta HSD (1FDT) | 3 (AC1-AC3) | AC3 | Gly9, Cys10, Sere11, Ser12, Gly13, Ile14, Gly15, Thr35, Leu36, Arg37, Leu64, Asp65, Val66, Arg67, Asn90, Ala91, Gly92, Thr140, Gly141, Tyr155, Lys159, Cys185, Gly186, Val188, Thr190, Phe192. |
| 2 | Cyclooxygenase -2 (4COX) | 24 (CAT,ACE,HEM, SUB, AC1-AC9, BC1-BC9, CC1-CC2) | BC2 | Arg120, Val349, Leu352, Ser353, Tyr355, Leu384, Tyr385, Trp387, Met522, Val253, Glu526, Ala527, Ser530, Leu531. |
| 3 | Dihydrofolate redcutase (4DFR) | 13 (APT,ANM, AAB, AGL, BPT, BNM, BAB, BGL, AC1-AC5) | APT | Ile5,Ala6, Ala7, Trp22, Asp27, Leu28, Phe31, Ile94, Thr113. |
| 4 | Exchange protein directly activated by CAMP (3CF6) | 3 (AC1-AC3) | AC2 | Phe367, Ile388, Cys395, Phe403, Gly404, Leu406, Arg414, Ala415, Leu449, Lys450, Glu451, Lys489. |
| 5 | Fructose 1,6 bisphophatase (2JJK) | 2 (AC1-AC2) | AC2 | Val17, Met18, Gly21, Arg22, Ala24, Gly26, Thr27, Gly28, Glu29, Leu30, Thr31, Met177. |
| 6 | Glucocorticoid receptor (1M2Z) | 5 (AC1-AC5) | AC4 | Leu563, Asn564, Gln570, Met601, Met604, Arg611, Phe623, Phe623, Gln642, Tyr735, Cys736, Phe749. |
| 7 | Glycogen synthase kinase (1q5k) | 2 (AC1-AC2) | AC1 | Ile62, Val70, Ala83, Leu132, Asp133, Tyr134, Val135, Pro136, Arg141, Leu188,. |
| 8 | Human acetylcholinesterase (4BDT) | 12 (AC1-AC9, BC-BC3) | AC1 | Trp86, Gly121, Gly122, Ser203, Tyr337, Trp439, His447,. |
| 9 | Human aldose reductase (3G5E) | 2 (AC1-AC2) | AC1 | Gly18, Thr19, Trp20, Lys21, Asp43, Tyr48, Lys77, His110,Trp11, Ser159, Asn160, Gln183, Tyr209, Ser210, Pro21, Leu212, Gly213, Ser214, Pro215, Asp216, Ala245, Ile260, Pro261, Lys262, Ser263, Val264, Thr265, Arg268, Glu271, Asn272. |

**Table 23 continued**

| Sl. No. | Target protein | Total No. of active sites | Best active site | Critical amino acid at selected active site |
|---|---|---|---|---|
| 10 | Human androgen receptor (1E3G) | 1 (AC1) | AC1 | Leu704, Asn705, Gln711, Met745, Met749, Arg752, Thr877. |
| 11 | Human beta secretase 1 (2XFJ) | 1 (AC1) | AC1 | Gly73, Gly74, Leu91, Asp93, Gly95, Ser96, Val130, Pro131, Tyr132, Thr133, Gln134, Phe169, Trp176, Ile179, Tyr259, Asp289, Gly291, Thr292, Thr293, Asn294,. |
| 13 | Human dipepetidyl peptidase IV (1X70) | 27 (AC1-AC9, BC-BC9, CC1-CC9) | CC8 | Arg125, Glu205, Glu206, Val207, Ser209, Phe357, Arg358, Tyr547. |
| 14 | Human estrogen receptor ligand binding domain (1ERR) | 2 (AC1-AC2) | AC1 | Leu346, Thr347, Ala350, Asp351, Glu353, Leu354, Trp383, Leu387, Arg394, Phe404, Met421, Ile424, His524, Leu525, Leu536. |
| 15 | Human glucokinase (1V4S) | 3 (AC1-AC3) | AC1 | Ser151, Phe152, Pro153, Thr168, Lys169, Asn204, Asp205, Ile225, Gly229, Cys230, Asn231, Glu256, Gln287, Glu290. |
| 16 | Human multidrug resistance protein (2CBZ) | 2 (AC1-AC2) | AC2 | Trp653, Thr660, Val680, Gly681, Cys682, Gly683, Lys684, Ser685, Ser686, Gln713, Lys764. |
| 17 | NAT-2 receptor (2PFR) | 34 (AC1-AC9, BC-BC9, CC1-CC9, DC1-DC7) | AC8 | Cys68, Ile95, Pro97, Val98, Thr103, Gly104, His107, Glu124, Tyr208, Thr214, Ser215, Ser216, Phe217, Ser287. |
| 18 | Nitric oxide synthase (4NOS) | 17 (COF, SUB, ZNB, AC1-AC9, BC1-BC5) | AC3 | Trp194, Arg199, Cys200, Gln205, Ser242, Phe369, Asn370, Trp372, Glu377, Trp463, Tyr489, Tyr491. |
| 19 | Phosphoinositide 3-kinase (1E8W) | 1 (AC1) | AC1 | Trp812, Lys833, Tyr867, Ile879, Glu880, Ile881, Val882, Met953, Ile963, Asp964. |
| 20 | Polo-like kinase1 (3KB7) | 3 (AC1-AC3) | AC1 | Leu59, Cys67, Ala80, Lys82, Leu130, Glue131, Cys133, Arg134, Arg136, Ser137, Glu140, Gly180, Phe183, Asp194. |

**Table 23 continued**

| Sl. No. | Target protein | Total No. of active sites | Best active site | Critical amino acid at selected active site |
|---|---|---|---|---|
| 21 | Pyruvate dehydrogenase kinase (4MP2) | 2 (AC1-AC2) | AC1 | Asn255, Ala259, Glu262, Asp290, Gly294, Val295, Leu303, Leu330, Leu346, Thr354. |
| 22 | Tau Protein Kinase 1 (1J1B) | 2 (AC1-AC2) | AC1 | Ile62, Asn64, Gly65, Phe67, Val70, Ala83, Lys85, Val110, Asp133, Tyr134, Val135, Arg141, Gln185, Asn186, Leu188, Asp200. |
| 23 | Vascular endothelial growth factor (3HNG) | 2 (AC1-AC2) | AC1 | Ly861, Glu878, Val907, Val909, Glu910, Tyr911, Cys912, Leu1013, Leu1029, Ile1038, Cys1039, Asp1040. |

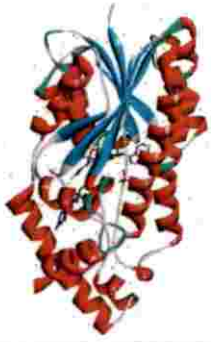
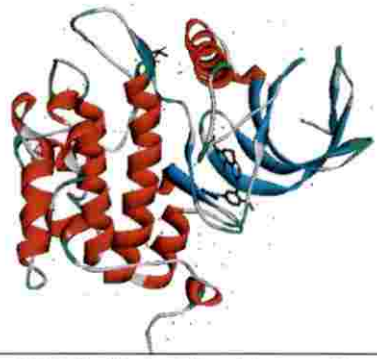
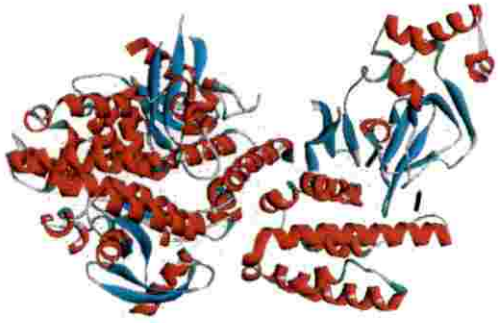
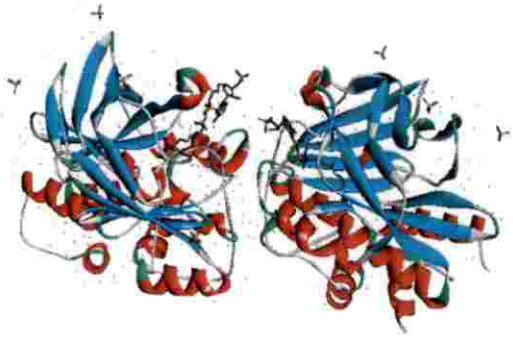
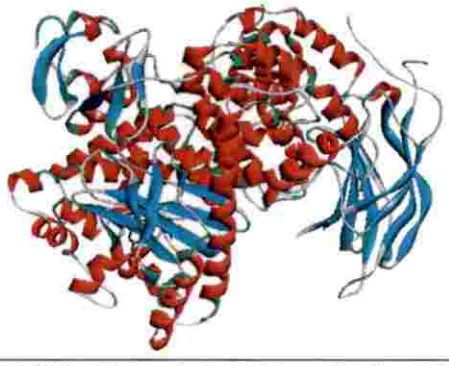
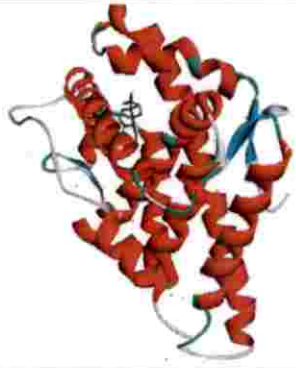
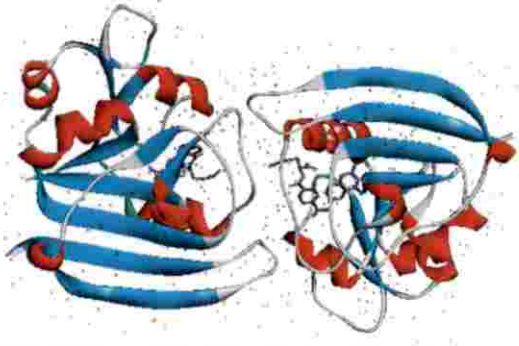
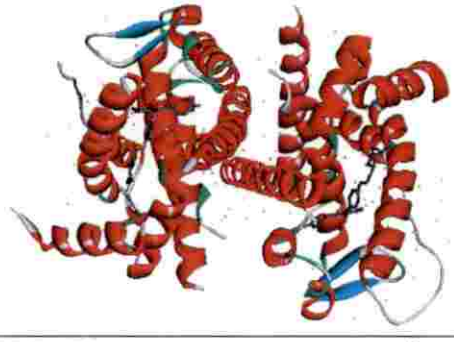
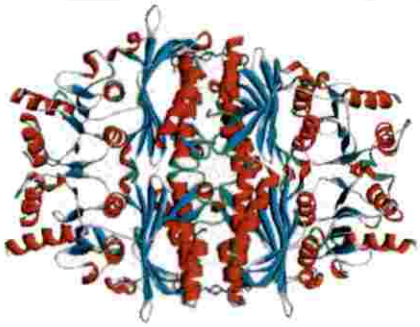
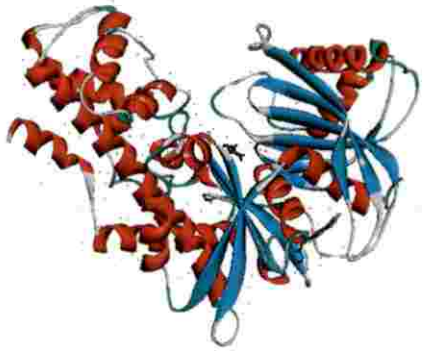
| | |
|---|---|
|  |  |
| 1FDT (17 beta-HSD) | 3KB7 (Polo-like kianse 1) |
|  |  |
| 3CF6 (Exchange protein directly ativated by CAMP) | 2PFR (NAT-2 receptor) |
|  |  |
| 1EW8 (Phosphoinotidate-3 kinase) | 1E3G (Human androgen receptor) |
|  |  |
| 4DFR (dihydrofolate reductase) | 1ERR (Human estrogen receptor ligand binding domain) |

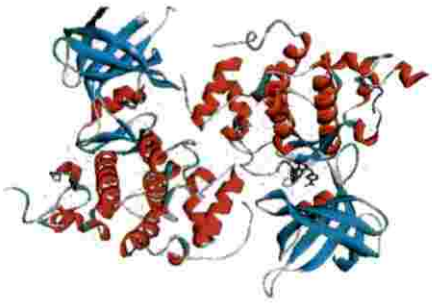
**Fig. 3: Structure of targets for cancer retrieved from protein databank**



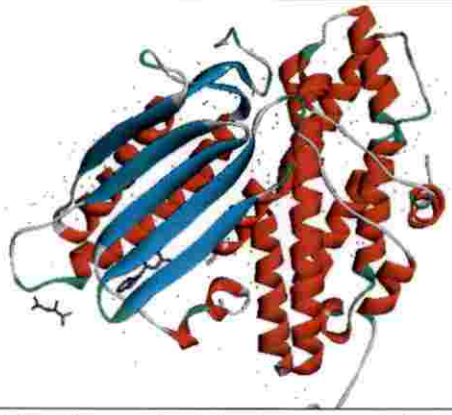
2JJK (Fructose 1,6-bisphosphatase)



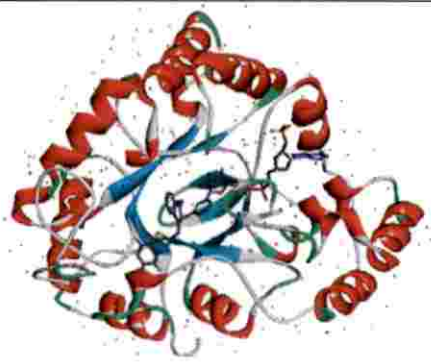
1V4S (Human Glucokinase)



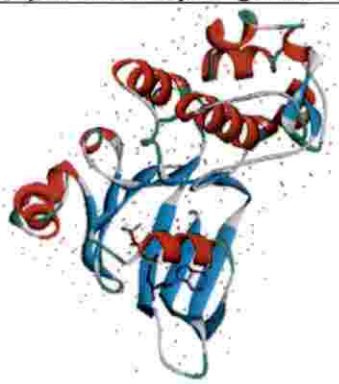
1Q5K (Glycogen synthase kinase)



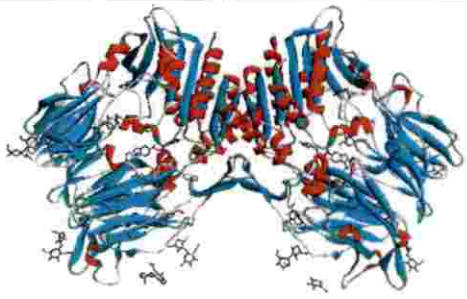
4MP2 (Pyruvate dehydrogenase kinase)



3G5E (Human aldose reductase)



2CBZ (Human multidrug resistance protein)



1X70 (Dipeptidyl peptidase IV)

**Fig. 4: Structure of targets for diabetes retrieved from protein databank**

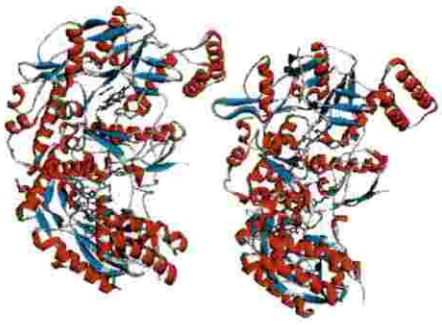
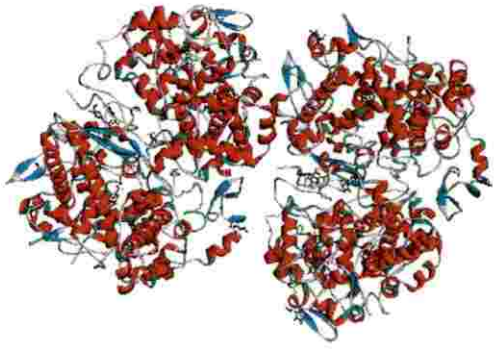
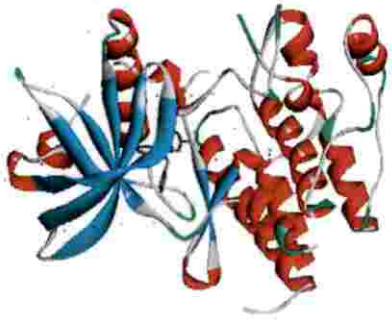
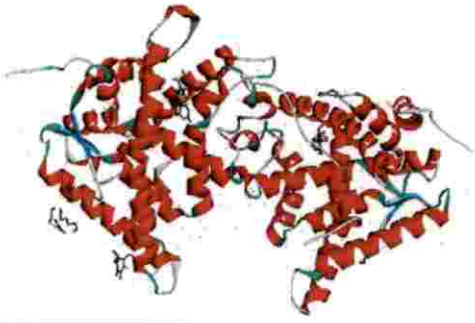
| | |
|---|---|
|  |  |
| 4NOS Nitric oxide synthase) | 4COX (Cyclooxygenase) |
|  |  |
| 3HNG (Vascular endothelial growth factor) | 1M2Z (Glucocorticoid receptor) |

**Fig. 5: Structure of targets for arthritis retrieved from protein databank**

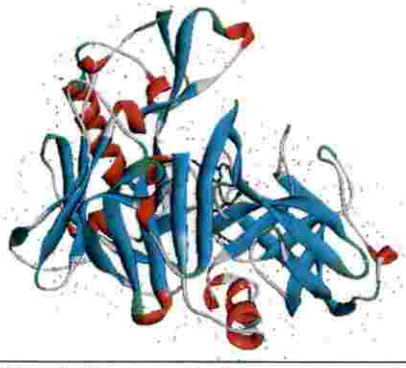
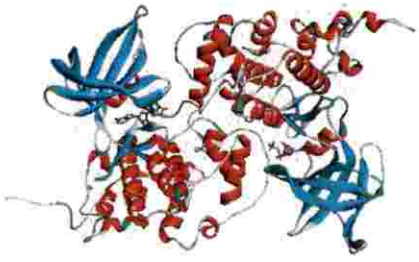
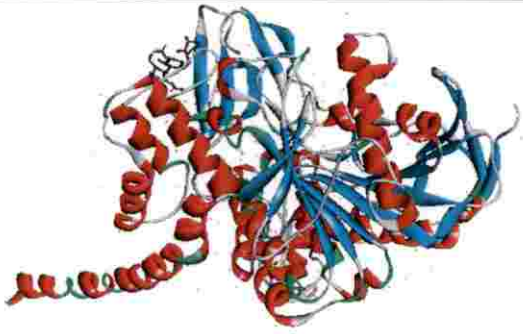
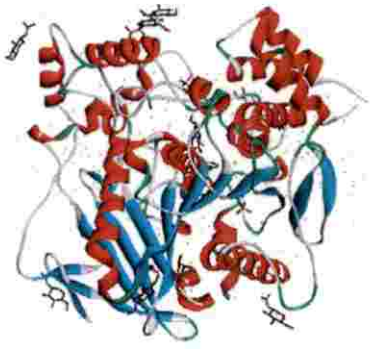
| | |
|---|---|
|  |  |
| 2XFJ (Human beta secretase 1) | 1J1B (Tau protein) |
| | |
|  |  |
| 4BDT (Human Acetylcholinesterase) | 4BDS (Human Butyrylcholinesterase) |

**Fig. 6: Structure of targets for Alzheimer's retrieved from protein databank**

maximum number of amino acid present in active site were 30 for human aldose reductase.

### 4.2.4 Molecular docking and analyses

Molecular docking was performed using 'CDOCKER' protocol in Discovery Studio between selected target and identified phytocompound. The FDA approved drugs were assessed using same protocol to do comparative study. Thus, pharmacological properties of curry leaf like anticancerous, antidiabetic, anti-inflammatory was assessed using CDOKCER protocol. Best ligand against various target related to above mentioned properties were identified on the basis of CDOCKER energy and CDOCKER interaction energy criteria by measuring difference between the both. Lesser the difference, better the interaction. The ligand with same CDOCKER energy and CODKCER interaction energy was considered as the best ligand. At same time, if difference between interaction energy is more than ten, binding between target and ligand becomes unstable. Such interactions were rejected. Other criteria like CDOCKER interaction energy should be higher than CDOCKER energy, No. of hydrogen bonds between target and ligand should be more and distance of hydrogen bond should be shorter were considered. Result of molecular docking is presented for the various diseases.

#### *4.2.4.1 Docking result of proteins associated with cancer*

##### *4.2.4.1.1 17β hydroxysteroid dehydrogenase (17-beta HSD)*

Docking results of 17-beta HSD with curry leaf ligands and FDA approved drugs are presented in Table 24. Total four compounds of curry and BHA interacted with target. Valylmethionine recorded best docking score for the target with binding energy of -66.7903 (kcal/mol), with difference of 0.0388 (kcal/mol) in CDOKCER energies (Fig.7). The compound interacted with target through four hydrogen bonds to active amino acids Ser12, Gly15, Asn90 and Lys159 with distance 2.02656, 2.00665, 2.12888 and 2.07639 Å respectively. The alpha-aminodiphenylacetic acid interacted with 17-beta HSD with very less binding energy (-12.3566 kcal/mol) compared to other compound interacted with same target.

BHA interacted with target (17-beta HSD) via three hydrogen bond with average binding energy (-30.1114 kcal/mol). Commercial drug used against 17-beta HSD failed to pass Lipinski rule of five hence rejected during docking.

**Table 24: Docking scores for the cancer target 17-Beta HSD with selected ligands**

| Type of compound | Sl. No. | Ligand | (-) CDOCKER energy (Kcal/mol) | (-) CDOCKER Interaction energy (Kcal/mol) | No. of H bonds | Amino acid bound to H bond | Distance (Å) | Binding Energy (Kcal/mol) |
|---|---|---|---|---|---|---|---|---|
| Curry leaf phytocompounds | 1 | Valylmethionine | 34.9551 | 34.9939 | 4 | Ser12*<br>Gly15*<br>Asn90*<br>Lys159* | 2.02656<br>2.00665<br>2.12888<br>2.07639 | -66.7903 |
| | 2 | Flucoxetine | 23.9072 | 33.6213 | 3 | Ser12*<br>Thr190(2)* | 2.07231<br>1.90962 | -26.1875 |
| | 3 | Prometon | 25.9838 | 28.5795 | 1 | Ser12* | 2.38996 | -19.8592 |
| | 4 | Alpha-aminodiphenylacetic acid | 23.1847 | 30.4697 | 5 | Lys159(2)*<br>Asn90(2)*<br>Gly9* | 2.37818<br>1.77962<br>2.19294 | -12.3566 |
| Standard antioxidant | 5 | BHA | 16.4736 | 24.9752 | 3 | Gly15*<br>Ser12*<br>Asn90* | 1.96752<br>1.93116<br>2.03604 | -30.1114 |
| Commercial drug | 6 | Fluvestrant | Failed in Lipinski Veber rule | | | | | |

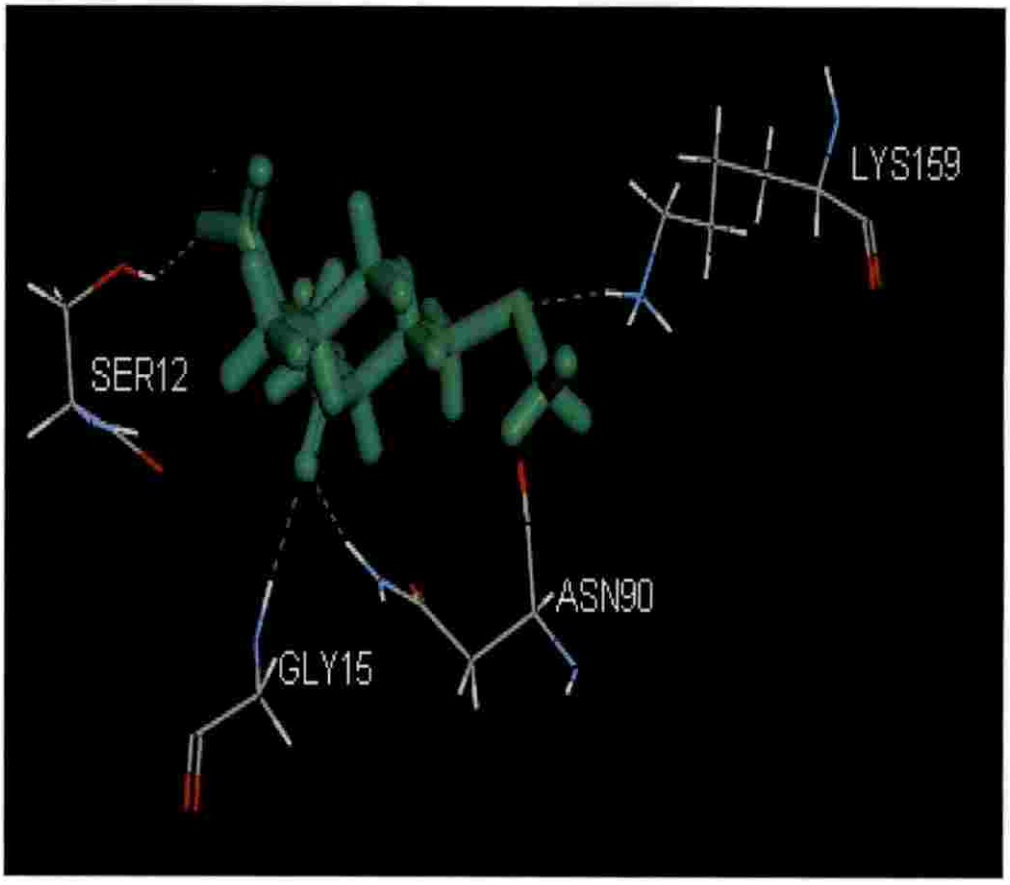



**Fig. 7: Hydrogen bond interaction of cancer target 17 beta-HSD (1FDT) with Valylmethionine**

#### *4.2.4.1.2 Polo-like kinase 1 (PLK)*

During molecular docking with polo-like kinase four phytocompounds interacted (Table 25). Amongst four, best docking score was recorded for valylmethonine with

-122.5233 kcal/mol of binding energy. It interacted to active amino acid residue Lys81 of the target (PLK) with distance of 1.98307 Å (Fig.8). Difference between CDOCKER energy and CDOCKER interaction energy was less (1.528 kcal/mol). Though, binding energy of Histidinol was less (-29.629 kcal/mol), the difference between CDOCKER energy and CDOCKER interaction energy was good (1.045 kcal/mol). All compounds interacted through single amino acid. Less distance between hydrogen bonds was observed for the ligand Pheniramine.

Interaction of BHA with the target was unstable due to a difference of more than 10 kcal/mol between CDOCKER energy and CDOCKER interaction energy. The commercial drug regociclib had descent binding energy (-73.3288 kcal/mol) but recorded positive CDOCKER energy (-1.32815 kcal/mol).

#### *4.2.4.1.3 Exchange protein directly activated by CAMP 2 (EPCA2)*

Two compounds from curry leaf showed good interaction with target EPAC2 (Table 26). Alpha-aminodiphenylacetic acid was found to be the best recording binding energy of -153.8738 kcal/mol with a deviation of 7.9769 kcal/mol between CDOCKER energy and CDOCKER interaction energy. The interacting active site residues were Gly404 and Lys489 with a length of 1.81371 and 1.96444 Å (Fig. 9). Though histidinol bound strongly (-110.9667 kcal/mol) with the least difference between CDOCKER energy and CDOCKER interaction energy (6.6457kcal/mol), it interacted to the non-critical amino acid. BHA interacted through one hydrogen bond of length 1.99432 Å to active amino acid Leu406. Diphenylamine, a commercial drug failed to interact with target as no hydrogen bond formation occurred between target and ligand during docking.

#### *4.2.4.1.4 N-Acetyltransferase-2 receptor (NAT-2)*

Results of molecular docking of NAT-2 with curry leaf phytocompounds is presented in Table 27. Alpha-aminodiphenylacetic acid recorded best docking score with least deviation in CDOCKER energy and CDOCKER interaction energy (4.971 kcal/mol) and good binding energy (-77.86.36 kcal/mol). The interacting active amino acid were Ser216 and Thr214 with hydrogen bond length of 2.3497 and 1.91833 Å



101

**Table 25: Docking scores for the cancer target polo-like kinase 1 with selected ligands**

| Type of compound | Sl. No. | Ligand | (-) CDOCKER energy (Kcal/mol) | (-) CDOCKER Interaction energy (Kcal/mol) | No. of H bonds | Amino acid bound to H bond | Distance (Å) | Binding Energy (Kcal/mol) |
|---|---|---|---|---|---|---|---|---|
| Curry leaf phytocompounds | 1 | Valylmethionine | 38.9120 | 40.8400 | 1 | Lys82* | 1.98307 | -122.5233 |
| | 2 | Pheniramine | 28.9006 | 37.2325 | 1 | Ser287* | 1.92422 | -94.2229 |
| | 3 | Alpha-Aminodiphenylacetic acid | 21.8506 | 27.2960 | 1 | Arg136* | 2.07521 | -38.8795 |
| | 4 | Histidinol | 24.4151 | 25.4601 | 1 | Cys133* | 2.23143 | -29.6290 |
| Standard antioxidant | 5 | BHA | 17.5853 | 28.2903 | 1 | Cys133* | 1.93877 | -36.4093 |
| Commercial drug | 6 | Ribociclib | -1.32815 | 55.2089 | 1 | Asp194* | 2.10151 | -73.3288 |

**Table 26: Docking scores for the cancer target exchange protein directly activated by CAMP with selected ligands**

| Type of compound | Sl. No. | Ligand | (-) CDOCKER energy (Kcal/mol) | (-) CDOCKER Interaction energy (Kcal/mol) | No. of H bonds | Amino acid bound to H bond | Distance (Å) | Binding Energy (Kcal/mol) |
|---|---|---|---|---|---|---|---|---|
| Curry leaf phytocompounds | 1 | Alpha-aminodiphenylacetic acid | 33.6452 | 41.6221 | 2 | GLY404*<br>Lys489* | 1.81371<br>1.96444 | -153.8738 |
| | 2 | Hisitidinol | 22.7564 | 29.5362 | 3 | His781<br>Thr914<br>Glu62 | 2.07356 | -110.966 |
| | 3 | Valylmethionine | 12.3313 | 21.8548 | 4 | Arg404*<br>Lys489* | 1.81371<br>1.96444 | -29.2632 |
| Standard antioxidant | 4 | BHA | 22.1629 | 30.2810 | 1 | Leu406* | 1.99432 | -42.9882 |
| Commercial drug | 5 | Diphenylamine | 19.0380 | 26.2256 | | - | - | - |

**Table 27: Docking scores for the cancer target Nat -2 receptor with selected ligands**

| Type of compound | Sl. No. | Ligand | (-) CDOCKER energy (Kcal/mol) | (-) CDOCKER Interaction energy (Kcal/mol) | No. of H bonds | Amino acid bound to H bond | Distance (Å) | Binding Energy (Kcal/mol) |
|---|---|---|---|---|---|---|---|---|
| Curry leaf phytocompounds | 1 | Pheniramine | 28.9006 | 37.2325 | 1 | Ser287* | 1.92422 | -94.2229 |
| | 2 | Alpha-aminodiphenylacetic acid | 26.9884 | 31.9594 | 4 | Ser216(3)*<br>Thr214* | 2.3497<br>1.91883 | -77.8636 |
| | 3 | Histidinol | 20.5092 | 27.1549 | 2 | Ser287*<br>Ser216* | 2.02809 | -56.9825 |
| Standard antioxidant | 4 | BHA | 20.0084 | 27.8699 | 1 | Ser287* | 1.96362 | -47.2294 |
| Commercial drug | 5 | NSC-54767 | 53.2944 | 59.4875 | 1 | Gly104* | 2.4337 | -142.4264 |

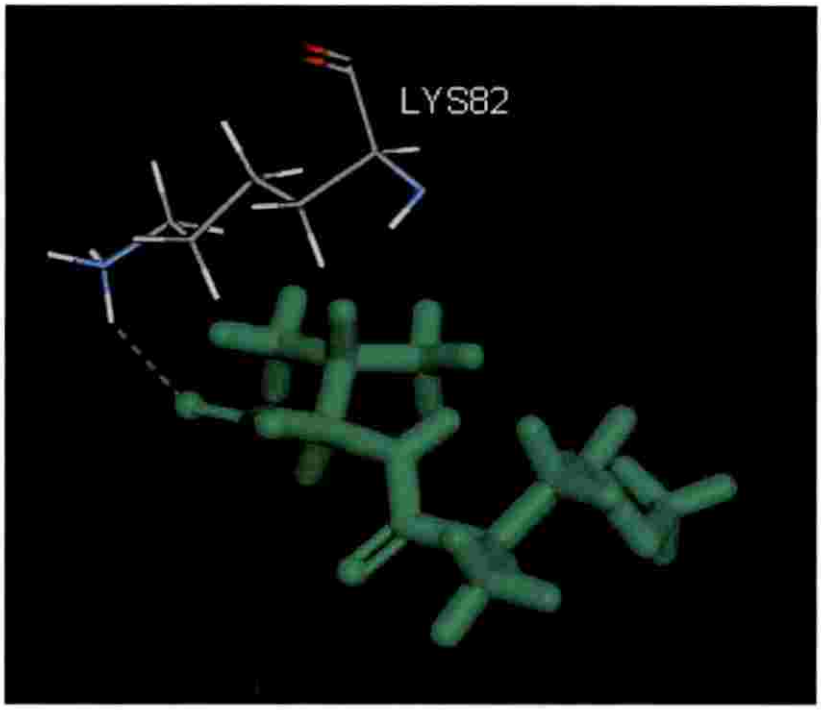



**Fig. 8: Hydrogen bond interaction of cancer target polo-like kianse 1 (3KB7) with Valylmethionine**

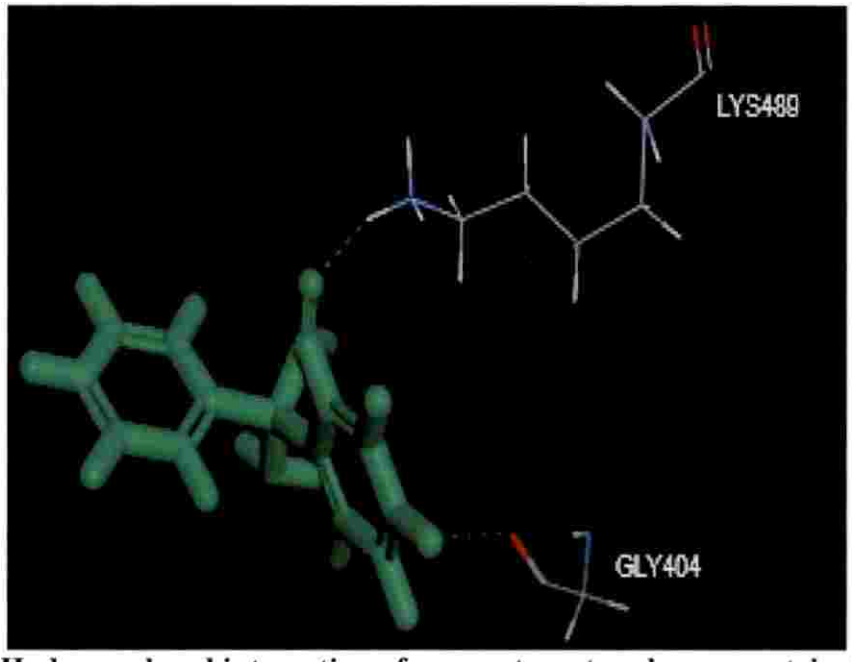



**Fig. 9: Hydrogen bond interaction of cancer target exchange protein directly ativated by CAMP (3CF6) with Alpha-aminodiphenylacetic acid**

respectively (Fig. 10). Pheniramine interacted strongly with target (-94.2229 kcal/mol) but there was high difference between CDOCKER energy and CDOCKER interaction energy (8.3391 kcal/mol). Shortest bond length was present for pheniramine and target (1.92422 Å).

NSC-54767, a drug used for inhibition of NAT-2 recorded best docking score among all interacted compounds with binding energy of -142.4264 kcal/mol, less than 10 kcal/mol deviation between CDOCKER energy and CDOCKER interaction energy as well as binding to active amino acid residue Gly104 with 2.4337 Å bond length.

#### *4.2.4.1.5 Phosphoinositide-3 kinase (PI3K)*

Three potent compounds *viz.* histidinol, pheniramine, and doxylamine interacted with target phosphoinositide-3 kinase (PI3K) during docking (Table 28). Histidinol was the best among all with less difference between CDOCKER energy and CDOCKER interaction energy (6.8306 kcal/mol) and binding energy of -96.1723 kcal/mol. It interacted with target through Glu880, critical amino acid in active site of PI3K by forming hydrogen bond of 2.29569 Å (Fig. 11). Interaction of pheniramine was weak with target as compared to histidinol due to increase in difference between CDOCKER interaction energies (8.1506 kcal/mol), binding through single amino acid (Met953) as well as low binding energy (-25.7181 kcal/mol). Another compound doxylamine interacted with target (PI3K) but at non-specific amino acid. The FDA approved drug failed to generate a single pose during molecular docking while standard antioxidant BHA interacted with target but was unable to form hydrogen bonds.

#### *4.2.4.1.6 Human Androgen receptor (AR)*

During docking of human androgen receptor with phytocompounds, one compound interacted with target (Table 29). Histidinol interacted with AR through 3 amino acids (Thr877, Asn705 and Leu704) in the active site with distance of 1.89568, 1.855509 and 2.0308 Å respectively (Fig. 12). The bonding energy scored was -137.0752 kcal/mol while deviation between CDOCKER interaction energies was 4.9072 kcal/mol. Overall Histidinol recorded best docking score.

Amongst commercial drugs for AR, nilutamid interacted with AR. The binding energy was -85.6514 kcal/mol while difference between CDOCKER energy was too high surpassing maximum limit. The interacting amino acid was Thr877. Another drug fenretinide interacted with positive docking energy. Synthetic antioxidant BHA

**Table 28: Docking scores for the cancer target phosphoinositide-3 kinase with selected ligands**

| Type of compound | Sl. No. | Ligand | (-) CDOCKER energy (Kcal/mol) | (-) CDOCKER Interaction energy (Kcal/mol) | No. of H bonds | Amino acid bound to H bond | Distance (Å) | Binding Energy (Kcal/mol) |
|---|---|---|---|---|---|---|---|---|
| Curry leaf phytocompounds | 1 | Histidinol | 23.7478 | 30.5784 | 3 | Glu880(2)*<br>Val882* | 2.29569<br>2.1777 | -96.1723 |
| | 2 | Pheniramine | 22.7752 | 30.9258 | 1 | Met953* | 2.28098 | -25.7181 |
| | 3 | Doxylamine | 22.4484 | 31.5954 | 1 | Ser806 | 2.01588 | -19.2485 |
| Standard antioxidant | 4 | BHA | 17.6706 | 25.5117 | - | - | - | - |
| Commercial drug | 5 | Tamoxifen | - | | | | | |

**Table 29: Docking scores for the cancer target human androgen receptor with selected ligands**

| Type of compound | Sl. No. | Ligand | (-) CDOCKER energy (Kcal/mol) | (-) CDOCKER Interaction energy (Kcal/mol) | No. of H bonds | Amino acid bound to H bond | Distance (Å) | Binding Energy (Kcal/mol) |
|---|---|---|---|---|---|---|---|---|
| Curry leaf phytocompounds | 1 | Histidinol | 28.3878 | 33.2952 | Thr877*<br>Asn705*<br>Leu704* | 3 | 1.89568<br>1.85509<br>2.03080 | -137.0752 |
| Standard antioxidant | 2 | BHA | 25.6846 | 34.7773 | Gln711* | 1 | 2.07177 | -37.4466 |
| Commercial drug | 3 | Nilutamide | 25.9272 | 43.1214 | Thr877* | 1 | 2.24698 | -85.6514 |
| | 4 | Fenretinide | Positive docking energy | | | | | |

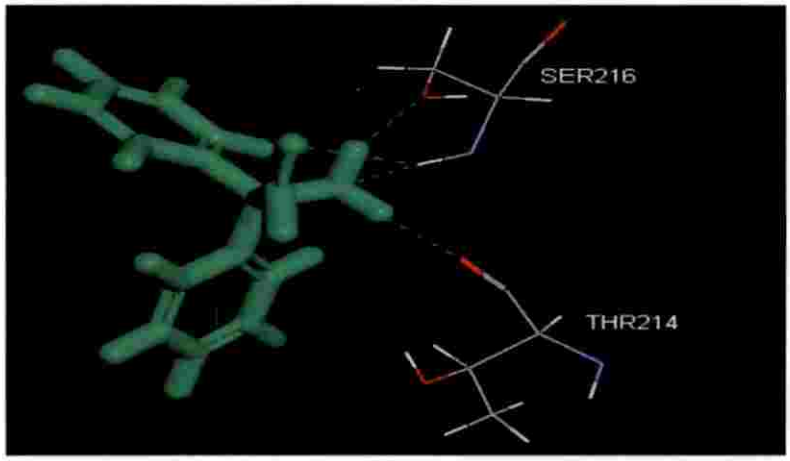



**Fig. 10: Hydrogen bond interaction of cancer target NAT-2 receptor (2PFR) with Alpha-aminodiphenylacetic acid**

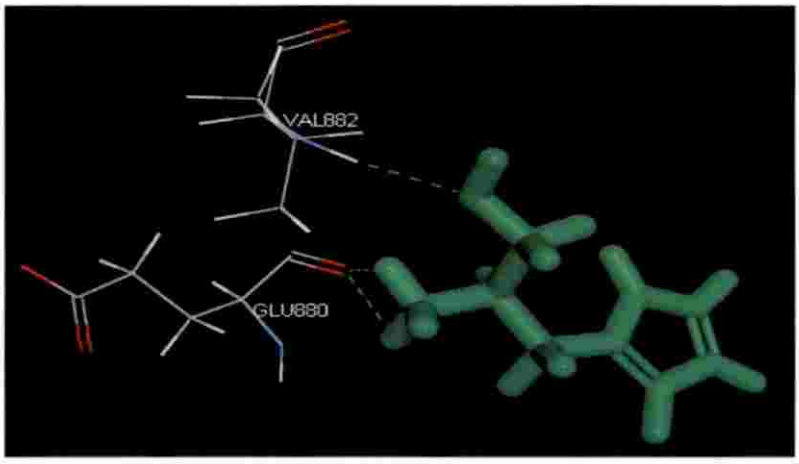



**Fig. 11: Hydrogen bond interaction of cancer target phosphoinotidate-3 kinase (1EW8) with Histidinol**

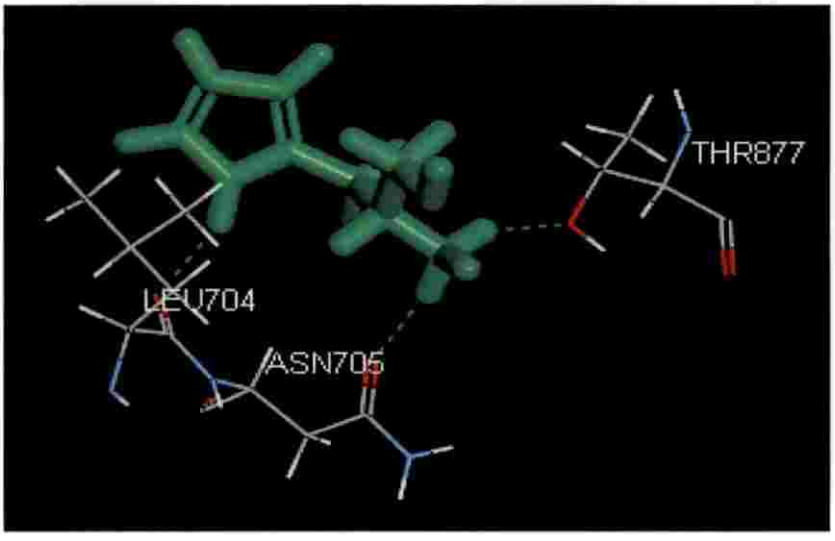



**Fig.12: Hydrogen bond interaction of cancer target human androgen receptor (1E3G) with Histidinol**

interacted well with target with binding of -37.4466 kcal/mol, 9.0827 kcal/mol difference between energy and CDOCKER interaction energy and bound to one amino acid Gln711 with 2.07177 Å bond length.

#### *4.2.4.1.7 Dihdryfolate reductase (DHFR)*

One compound from curry leaf interacted with target during molecular docking (Table 30). Histidinol bound to target with good docking score with difference of 4.4612 kcal/mol in DOCKER energy and CDOCKER interaction energy and binding energy of -61.0572 kcal/mol. The amino acids involved in interactions were Ile5 and Asp27 with 2.08063 and 1.91422 Å bond length respectively (Fig. 13). Interaction of BHA was normal with binding energy of -46.451 kcal/mol and deviation of 8.2924 kcal/mol between CDOCKER energy and CDOCKER interaction energy. The FDA drug trimetrexate interacted with high binding energy of -132.5889 kcal/mol through five amino acids (Gly, Ile5, Ile94 and Asp27 (2)). The length of hydrogen bonds varied between 1.96556 to 2.25951 Å. But, the difference between CDOCKER energy and CDOCKER interaction energy was beyond maximum limit required for stable confirmation.

#### *4.2.4.1.8 Human estrogen receptor ligand-binding domain*

Molecular docking result of human estrogen receptor ligand-binding domain with curry leaf phytocompound is presented in Table 31. One compound, Histidinol showed best interaction with target. Binding energy recorded was -233.3435 kcal/mol while difference between CDOCKER energy and CDOCKER interaction energy was 6.49 kcal/mol. It interacted with two amino acids in active site Leu346 and Glu353 (Fig. 14). BHA recorded the least binding energy amongst all ligands (-10.8 kcal/mol) and difference between CDOCKER energy and CDOCKER interaction energy was also surpassing maximum limit of 10 kcal/mol. On other hand, commercial drug, trimetrexate interacted with high binding energy (-161.2334 kcal/mol) and also hydrogen bond length was the least amongst all (1.999583 Å) but the difference between CODCKER energy and CDOCKER interaction energy crossed its maximum limit.

### *4.2.4.2 Docking result of proteins (targets) associated with diabetes*

#### *4.2.4.2.1 Fructose 1, 6-bisphosphatse (FBpase)*

In the process of docking fructose 1, 6-bisphosphatse with phytocompounds from curry leaf, four compounds interacted with target (Table 32). Interestingly, three

**Table 30: Docking scores for the cancer target dihydropholate reductase with selected ligands**

| Type of compound | Sl. No. | Ligand | (-) CDOCKER energy (Kcal/mol) | (-) CDOCKER Interaction energy (Kcal/mol) | No. of H bonds | Amino acid bound to H bond | Distance (Å) | Binding Energy (Kcal/mol) |
|---|---|---|---|---|---|---|---|---|
| Curry leaf phytocompounds | 1 | Histidinol | 24.4062 | 28.9232 | Ile5*<br>Asp27(2)* | 3 | 2.08063<br>1.91422 | -61.0572 |
| Standard antioxidant | 2 | BHA | 18.3893 | 26.6817 | Trp22* | 1 | 2.25661 | -46.452 |
| Commercial drug | 3 | Trimetrexate | 23.2455 | 57.0153 | Gly15<br>Ile5*<br>Ile94*<br>Asp27(2)* | 5 | 2.22369<br>1.96387<br>1.96556<br>2.25951 | -132.5889 |

**Table 31: Docking scores for the cancer target Human estrogen receptor ligand-binding domain with selected ligands**

| Type of compound | Sl. No. | Ligand | (-) CDOCKER energy (Kcal/mol) | (-) CDOCKER Interaction energy (Kcal/mol) | No. of H bonds | Amino acid bound to H bond | Distance (Å) | Binding Energy (Kcal/mol) |
|---|---|---|---|---|---|---|---|---|
| Curry leaf phytocompounds | 1 | Histidinol | 22.1024 | 28.5924 | Leu346*<br>Glu353* | 2 | 2.12417<br>2.22719 | -233.3435 |
| Standard antioxidant | 2 | BHA | 20.7126 | 30.0847 | Glu353* | 1 | 2.00878 | -10.8 |
| Commercial drug | 3 | Trimetrexate | 17.6477 | 56.9947 | Asp351* | 1 | 1.99583 | -161.2334 |

**Table 32: Docking scores for the diabetes target fructose-1, 6-bisphosphatase with selected ligands**

| Type of compound | Sl. No. | Ligand | (-) CDOCKER energy (Kcal/mol) | (-) CDOCKER Interaction energy (Kcal/mol) | No. of H bonds | Amino acid bound to H bond | Distance (Å) | Binding Energy (Kcal/mol) |
|---|---|---|---|---|---|---|---|---|
| Curry leaf phytocompounds | 1 | Alpha-aminodiphenylacetic acid | 21.7594 | 28.2431 | 2 | Gly26(2)* | 1.95478 | -117.7149 |
| | 2 | Valylmethionine | 31.4617 | 33.3619 | 1 | Thr31* | 2.4946 | -81.143 |
| | 3 | Doxylamine | 20.7181 | 27.9738 | 2 | Gly26(2)* | 2.12725 | -35.5302 |
| | 4 | Histidinol | 19.2569 | 24.1097 | 1 | Gly26* | 1.8825 | -111.9123 |
| Standard antioxidant | 5 | BHA | 15.7131 | 25.6175 | - | - | - | - |

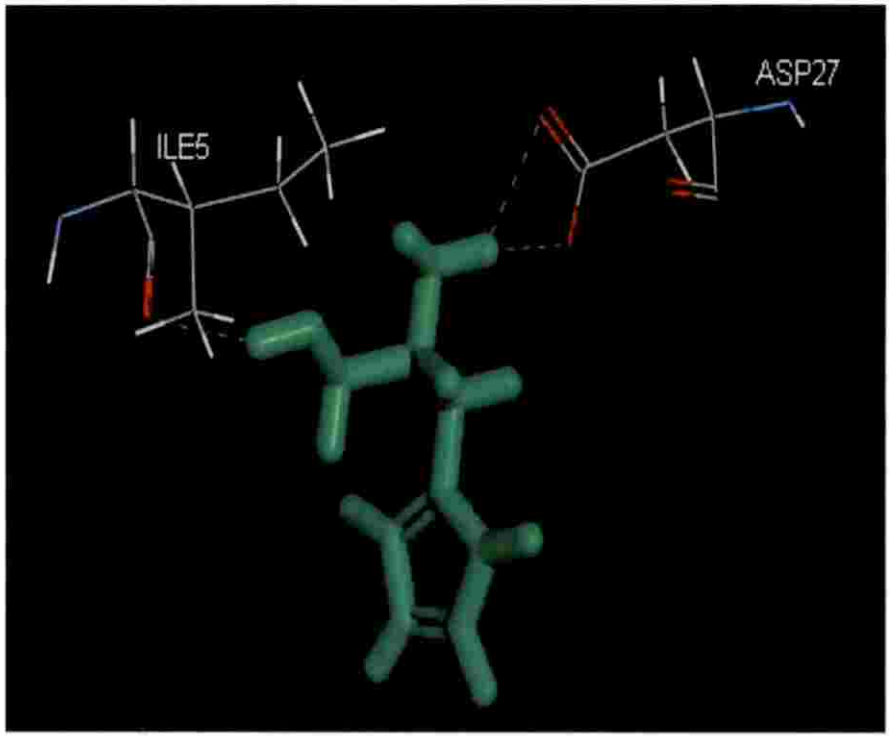



**Fig.13: Hydrogen bond interaction of cancer target dihydropholate reductase (4DFR) with Histidinol**

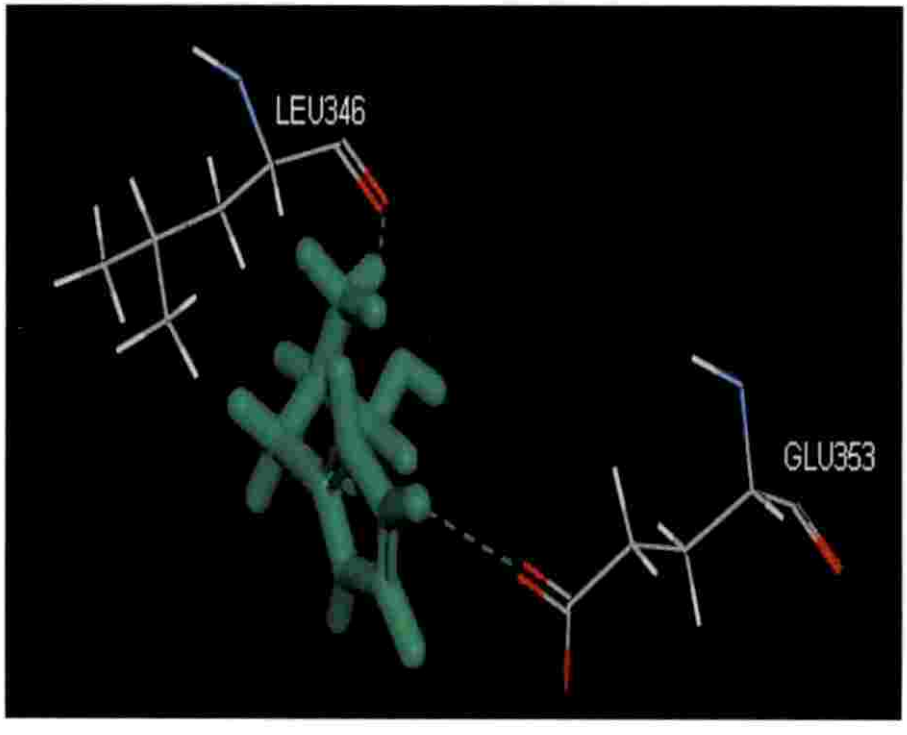



**Fig. 14: Hydrogen bond interaction of cancer target human estrogen receptor ligand binding domain (1ERR) with Histidinol**

out of four compounds interacted with target via critical amino acid in active site Glu26 while valylmethonine interacted with target (FBpase) via Thr31 another active amino acid. Valylmethonine recorded least difference between CDOCKER energy and CDOCKER interaction energy (1.9002 kcal/mol) followed by histidinol, doxylamine and alpha-aminodiphenylacetic acid. Valylmethoine recorded best docking score amongst all with binding energy of -81.143 kcal/mol interacted via active amino acid Thr31 (Fig. 15). The drug against the target are under trails and BHA failed to form hydrogen bond with target.

***4.2.4.2.2 Human glucokinase***

Molecular docking result of phytocompounds with target (human glucokinase) are presented in Table 33. DL-2-aminooctanoic acid recorded a best docking score during interaction with target. It recorded less difference between CDOCKER energy and CDOCKER interaction energy (0.2354 kcal/mol), high binding energy (-103.6547 kcal/mol) and interacted via Gly229 of 2.13189 Å in length (Fig. 16). Histidinol and prometon formed highest number of hydrogen bonds (4 No. each) followed by DL-2-aminooctanoic acid (2). BHA interacted with target but failed to form any hydrogen bond.

***4.2.4.2.3 Glycogen synthase kinase-3 (GSK3)***

Molecular docking studies of glycogen synthase kinase-3 revealed that four compounds from curry leaf interacted with target (Table 34). One compound valylmethionine scored the best docking score for the target. It interacted with target binding energy of -52.0888 kcal/mol also the difference between CDOCKER energy and CDOCKER interaction energy was least (1.218 kcal/mol). The interacting amino acids were Tyr134 and Val135 with hydrogen bond length of 2.45721 and 2.32348 Å respectively (Fig. 17). Highest docking energy for the target had been observed in interactions of target with flucoxetine (-65.9137 kcal/mol). Histidinol interacted with target via highest number of amino acids (4) followed by valylmethonine, flucoxetine, pheniramine.

Lithium carbonate, commercial drug used against GSK-3 recorded the least difference between CDOCKER energy and CDOCKER interaction energy (0.7024 kcal/mol) but binding energy was too low (-11.3106 kcal/mol). BHA interaction was average with target (-28.1772 kcal/mol binding energy, interaction via one amino acid, 8.4865 kcal/mol difference between CDOCKER energy and CDOCKER interaction energy).

**Table 33: Docking scores for the diabetes target human glucokinase with selected ligands**

| Type of compound | Sl. No. | Ligand | (-) CDOCKER energy (Kcal/mol) | (-) CDOCKER Interaction energy (Kcal/mol) | No. of H bonds | Amino acid bound to H bond | Distance (Å) | Binding Energy (Kcal/mol) |
|---|---|---|---|---|---|---|---|---|
| Curry leaf phytocompounds | 1 | Histidinol | 38.8288 | 42.9625 | 3 | Asp205(3)* | 1.95102 | -108.9567 |
| | 2 | DL-2-aminooctanoic acid | 39.4208 | 39.6562 | 3 | Gly229(2)* | 2.13189 | -103.6547 |
| | 3 | Prometon | 27.8147 | 31.1542 | 4 | Lys169*<br>Asn204*<br>Cys230*<br>Glu290* | 1.95357<br>2.2174<br>2.29548<br>2.03681 | -30.4201 |
| Standard antioxidant | 4 | BHA | -22.9359 | 13.8405 | - | - | - | - |

**Table 34: Docking scores for the diabetes target glycogen synthase kinase-3 with selected ligands**

| Type of compound | Sl. No. | Ligand | (-) CDOCKER energy (Kcal/mol) | (-) CDOCKER Interaction energy (Kcal/mol) | No. of H bonds | Amino acid bound to H bond | Distance (Å) | Binding Energy (Kcal/mol) |
|---|---|---|---|---|---|---|---|---|
| Curry leaf phytocompounds | 1 | Valylmethionine | 32.3976 | 33.4594 | 2 | Tyr134*<br>Val135* | 2.45721<br>2.32348 | -52.0888 |
| | 2 | Histidinol | 22.4151 | 31.1008 | 4 | Tyr136*(2)<br>Glu137<br>Val135* | 2.00938<br>2.44544<br>1.84022 | -7.3484 |
| | 3 | Flucoxetine | 22.6406 | 34.8718 | 1 | Tyr134* | 2.19566 | -65.9137 |
| | 4 | Pheniramine | 23.7827 | 33.4168 | 1 | Asn186 | 2.02809 | -64.9304 |
| Standard antioxidant | 5 | BHA | 19.0451 | 27.5361 | 1 | Asp133* | 2.26302 | -28.1772 |
| Commercial drug | 6 | Lithium carbonate | 10.9895 | 11.6919 | 1 | Val135* | 2.22809 | -11.3106 |

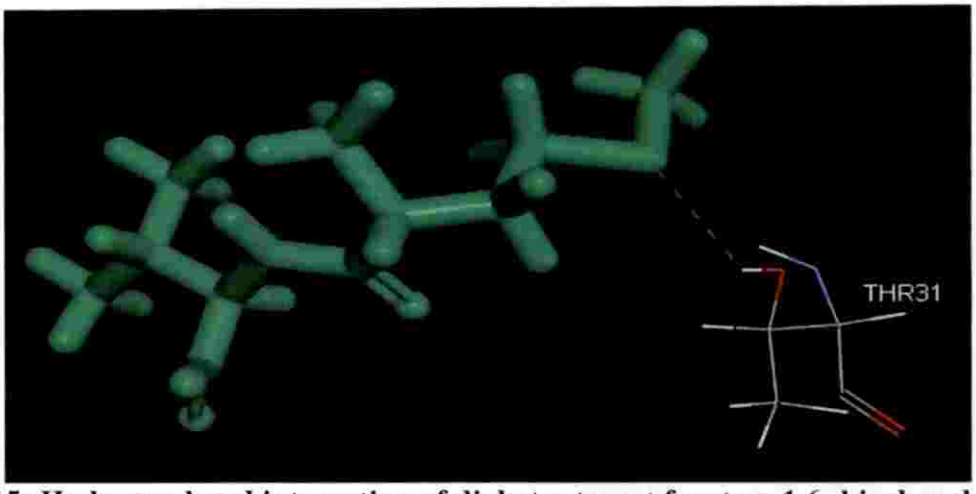



**Fig. 15: Hydrogen bond interaction of diabetes target fructose 1,6- bisphosphatase (2JJK) with with Valylmethionine**

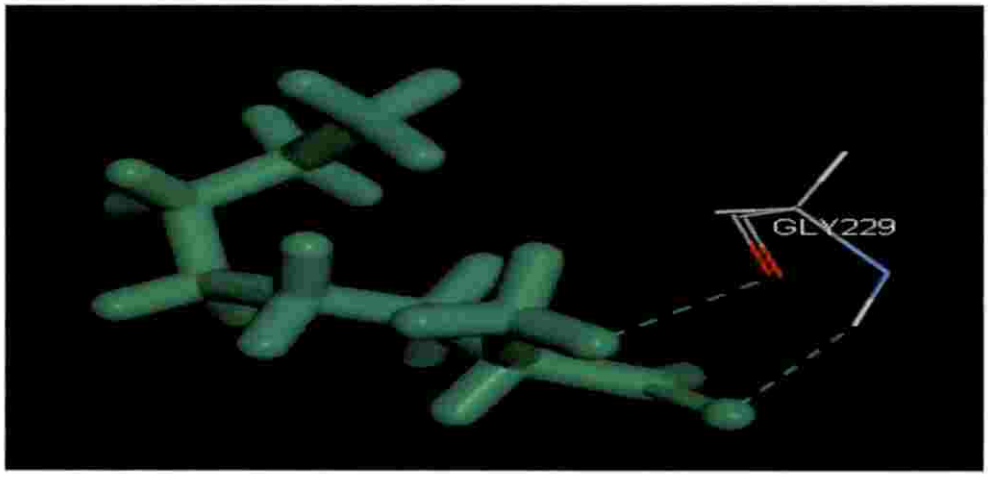



**Fig. 16: Hydrogen bond interaction of diabetes target human glucokinase (1V4S) with DL-2-aminooctanoic acid**

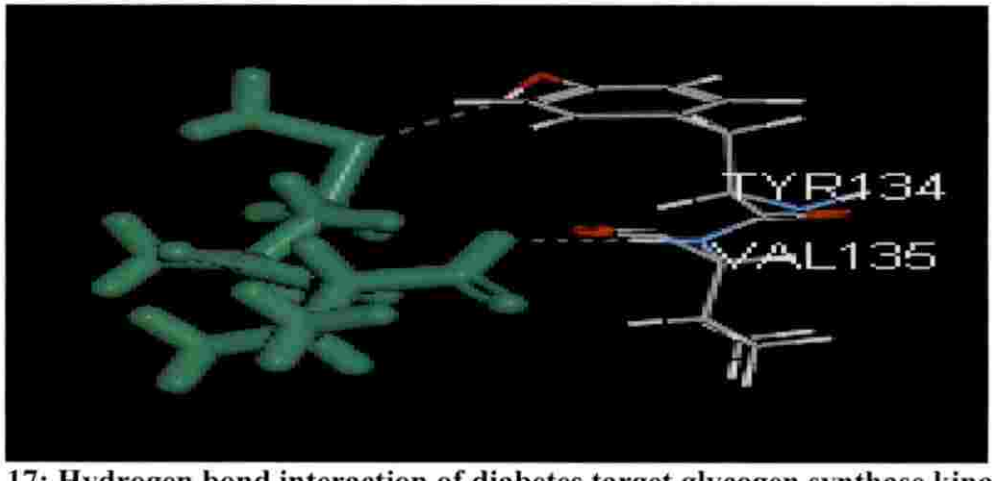



**Fig. 17: Hydrogen bond interaction of diabetes target glycogen synthase kinase-3 (1Q5K) with Valylmethionine**

#### *4.2.4.2.4 Pyruvate dehydrogenase kinase (PDK)*

With pyruvate dehydrogenase kinase, flucoxetine recorded best docking score. It interacted with good binding energy (-95.092 kcal/mol),) among all phytocompounds of curry leaf followed by alpha-aminodiphenylacetic acid, flucoxetine and valylmethonine (Table 35). The amino acid involved in interaction were Asn255 and Glu262 (Fig. 18). Valylmethionine and histidinol had less difference between CDOCKER energy and CDOCKER interaction energy but were interacting via non-critical amino acid. flucoxetine interacted via active amino acid in binding site Asn255 and Gly262 by forming hydrogen bond (2.41087 and 2.36659 Å). Though, BHA interacted with target but failed to form any hydrogen. The commercial drug, dichloroacetic acid recorded highest binding energy (-135.2625 kcal/mol) and least difference between CDOCKER energy and CDOCKER interaction energy (2.5606 kcal/mol) but interacted with non-critical amino acid in active site.

#### *4.2.4.2.5 Human aldose reductase (HAR)*

During docking of human aldose reducatse, two compounds from curry leaf interacted (Table 36). The alpha-aminodiphenylacetic acid scored best docking score with -130.945 kcal/mol of binding energy and interacted with target via active amino acid Ser210 by forming hydrogen bond of 2.20186 Å (Fig. 19). But, the deviation between CDOCKER energy and CDOCKER interaction energy was slightly higher (9.4955 kcal/mol). On other hand valylmethonine interacted with target with good docking score (-82.8526 kcal/mol and difference of 2.8905 kcal/mol between CDOCKER energy and CDOCKER interaction energy) but interacted with non-critical amino acid Asp139.

BHA interacted with target with -40.5216 kcal/mol binding energy and difference of 11.9023 kcal/mol between CDOCKER energy and CDOCKER interaction energy but interacted via non-crttical amino acid Cys298. The commercial drug, epalrestat recorder highest binding energy (-172.446 kcal/mol) via active amino acid Lys262 in biding site.

#### *4.2.4.2.6 Human multidrug resistance protein 1 (MDRP)*

Two compounds of curry leaf (Histidinol and Valylmethionine) interacted with human multidrug resistance protein 1 (Table 37). Histidinol interacted via Gln713 by forming hydrogen bond of 1.89254 Å with binding energy of -91.5718 kcal/mol (Fig. 20). Valylmethionine recorded less difference between CDOCKER energy and

**Table 35: Docking scores for the diabetes target pyruvate dehydrogenase kinase with selected ligands**

| Type of compound | Sl. No. | Ligand | (-) CDOCKER energy (Kcal/mol) | (-) CDOCKER Interaction energy (Kcal/mol) | No. of H bonds | Amino acid bound to H bond | Distance (Å) | Binding Energy (Kcal/mol) |
|---|---|---|---|---|---|---|---|---|
| Curry leaf phytocompounds | 1 | Alpha-aminodiphenylacetic acid | 23.8142 | 33.0193 | 1 | Thr354* | 2.00915 | -22.3412 |
| | 2 | Flucoxetine | 30.6012 | 39.3762 | 2 | Asn255*<br>Glu262* | 2.41087<br>2.36659 | -95.0920 |
| | 3 | Histidinol | 24.7137 | 32.1108 | 1 | Met288 | 1.84929<br>2.19232 | -118.8296 |
| | 4 | Valylmethionine | 33.9556 | 36.2832 | 1 | Arg258 | 2.1228 | -16.9073 |
| Standard antioxidant | 5 | BHA | 4.99132 | 13.3287 | - | - | - | - |
| Commercial drug | 6 | Dichloroacetic acid | 14.4027 | 16.9633 | 1 | Arg380 | 1.94585 | -133.2625 |

**Table 36: Docking scores for the diabetes target human aldose reductase with selected ligands**

| Type of compound | Sl. No. | Ligand | (-) CDOCKER energy (Kcal/mol) | (-) CDOCKER Interaction energy (Kcal/mol) | No. of H bonds | Amino acid bound to H bond | Distance (Å) | Binding Energy (Kcal/mol) |
|---|---|---|---|---|---|---|---|---|
| Curry leaf phytocompounds | 1 | Alpha-aminodiphenylacetic acid | 25.7286 | 35.2241 | 1 | Ser210* | 2.20186 | -130.9450 |
| | 2 | Valylmethionine | 23.7509 | 24.6413 | 1 | Asp139 | 2.169 | -82.8526 |
| Standard antioxidant | 3 | BHA | 17.5744 | 29.4767 | 1 | Cys298 | 2.1643 | -40.5261 |
| Commercial drug | 4 | Epalrestat | 16.0595 | 45.6787 | 1 | Lys262* | 2.01991 | -172.4446 |

**Table 37: Docking scores for the diabetes target human multidrug resistance protein with selected ligands**

| Type of compound | Sl. No. | Ligand | (-) CDOCKER energy (Kcal/mol) | (-) CDOCKER Interaction energy (Kcal/mol) | No. of H bonds | Amino acid bound to H bond | Distance (Å) | Binding Energy (Kcal/mol) |
|---|---|---|---|---|---|---|---|---|
| Curry leaf specific | 1 | Histidinol | 16.512 | 21.1078 | 4 | Tyr 710(2)<br>Gln713*<br>Asp792 | 2.35952<br>1.89264<br>1.9414 | -91.5718 |
| | 2 | Valylmethionine | 18.0829 | 20.8054 | 1 | Lys684* | 2.07576 | -24.4517 |
| Standard antioxidant | 3 | BHA | 9.3901 | 17.6779 | 5 | Lys684(5)* | 2.34209 | -12.9807 |
| | 3 | Polaprezinc | 42.8588 | 36.212 | 1 | Lys684* | 2.48494 | -47.4599 |
| Commercial drug | 4 | Technetium Tc-99m medronate | 55.528 | 53.3902 | 5 | Lys684(3)*<br>Ser685 | 2.21307<br>1.89442 | -249.2964 |

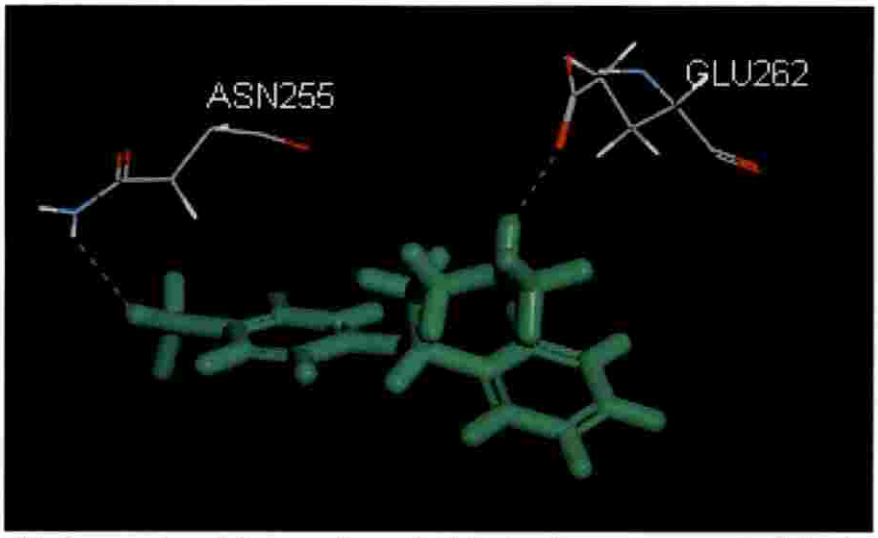



**Fig. 18: Hydrogen bond interaction of diabetes target pyruvate dehydrogenase kinase (4MP2) with Flucoxetine**

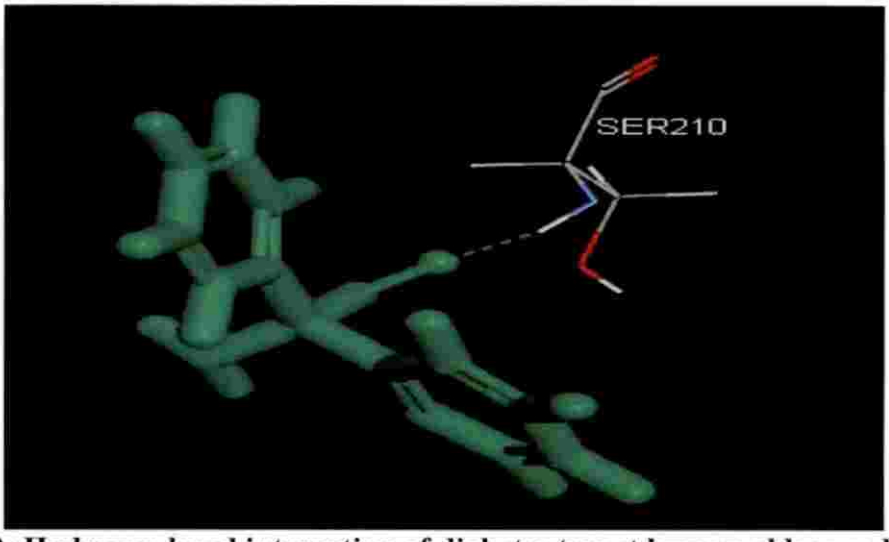



**Fig. 19: Hydrogen bond interaction of diabetes target human aldose reductase (3G5E) with Alpha-aminodiphenylacetic acid**

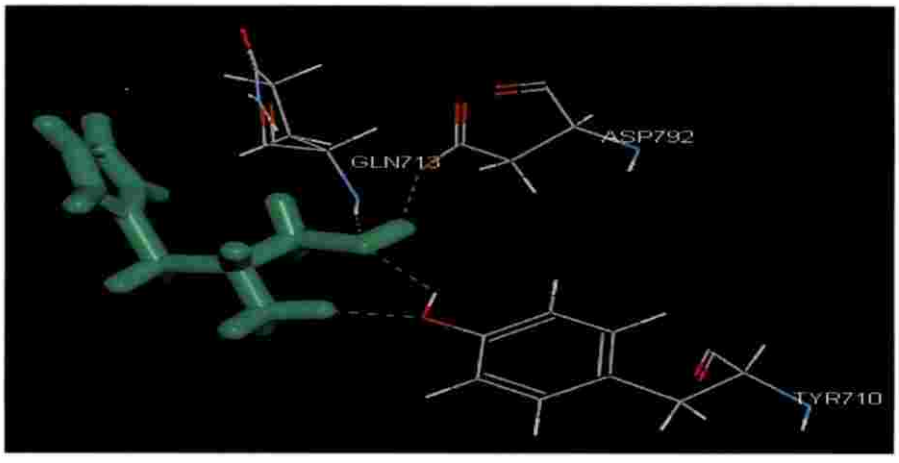



**Fig. 20: Hydrogen bond interaction of diabetes target human multidrug resistance protein (2CBZ) with Histidinol**

CDOCKER interaction energy (2.07576 kcal/mol) and with binding energy of -82.8526 kcal/mol. Commercial drug, technetium Tc-99m medronate bonded strongly to target (-249.2964 kcal/mol binding energy). But both drugs showed higher CDOCKER energy (55.528 kcal/mol) than CDOCKER interaction energy (53.3902 kcal/mol).

#### *4.2.4.2.7 Human dipeptidyl peptidase-IV (DPP-IV)*

Molecular docking results of Human dipeptidyl peptidase-IV with phytocompounds and FDA approved drugs are presented in Table 38. Among phytocompounds of curry leaf, histidinol interacted with the target with maximum number of hydrogen bonds (4) followed by alpha-aminodiphenylacetic acid, norpropoxyphene and pheniramine. Histidinol scored good docking score during interaction with target. It recorded highest binding energy amongst all (-240.3181 kcal/mol). The interacting amino acids were Glu205, Glu206 which formed hydrogen bonds of length 1.71098 and 1.82808 Å respectively (Fig. 21). Smallest hydrogen bond length amongst phytocompounds was formed between target and Histidinol (1.71098 Å) while largest bond length recorded was with Valylmethionine (2.44221 Å). Overall, histidinol showed best docking score.

Among commercial drug, myrcetin interacted with good binding energy (-145.3586 kcal/mol) via four active amino acids. Apigenin recorded less deviation between CDOCKER energy and CDOCKER interaction energy (-1.5685 kcal/mol). Standard antioxidant BHA interacted with target via 2 critical amino acids (Glu206 and Arg125) with binding energy of -58.4061 kcal/mol.

### *4.2.4.3 Docking result of proteins (targets) associated with arthritis*

#### *4.2.4.3.1 Nitric oxide synthase (NOS)*

With nitric oxide synthase, four phytocompounds interacted during molecular docking (Table 39). Histidinol recorded best docking score. It interacted with binding energy of -109.4131 kcal/mol. Histidinol interacted to target by binding to three active amino acid in binding site (Trp372, Glu377 and Arg199) via hydrogen bonds (Fig. 22). Norproproxyphene and flucoxetine form two hydrogen bonds while single bond was formed between target and pheniramine. Amongst all, histidinol recorded good docking score with -109.5131 binding energy, less deviation between CDOCKER energy and CDOCKER interaction energy (6.5135 kcal/mol) and three hydrogen bonds.

The commercial drug, apigenin recorded least difference between CDOKCER energies (1.2919 kcal/mol) amongst all interacted compounds. It interacted with

**Table 38: Docking scores for the diabetes target human dipeptidyl peptidase IV with selected ligands**

| Type of compound | Sl. No. | Ligand | (-) CDOCKER energy (Kcal/mol) | (-) CDOCKER Interaction energy (Kcal/mol) | No. of H bonds | Amino acid bound to H bond | Distance (Å) | Binding Energy (Kcal/mol) |
|---|---|---|---|---|---|---|---|---|
| Curry leaf phytocompounds | 1 | Histidinol | 37.3715 | 44.0897 | 4 | Glu205(2)*<br>Glu206*<br>Tyr662 | 1.71098<br>1.82805<br>2.12245 | -240.3784 |
| | 2 | Norpoproxyphene | 31.9798 | 41.9349 | 2 | Glu206*<br>Glu205* | 1.9466<br>2.16599 | -146.9897 |
| | 3 | Alpha-aminodiphenylacetic acid | 26.3799 | 33.3558 | 3 | Tyr662<br>Arg125*<br>Asn710 | 2.25122<br>1.9346<br>2.36915 | -9.954 |
| | 4 | Pheniramine | 30.9386 | 36.1647 | 1 | Tyr662 | 1.83085 | -133.6718 |
| | 5 | Valylmethionine | 32.8405 | 33.3055 | 2 | Arg125*<br>Glu205* | 1.9188<br>2.3339 | -6.1518 |
| Standard antioxidant | 6 | BHA | 14.1151 | 22.5475 | 2 | Arg125*<br>Glu206* | 2.44221<br>1.97955 | -58.4061 |
| Commercial drug | 7 | Apigenin | 34.5819 | 36.1504 | 1 | Arg125* | 1.70479 | -32.8294 |
| | 8 | Myricetin | 35.1728 | 42.554 | 4 | Arg125(3)*<br>Glu205* | 1.98466<br>2.02654 | -145.3386 |

**Table 39: Docking scores for the arthritis target nitric oxide synthase with selected ligands**

| Type of compound | Sl. No. | Ligand | (-) CDOCKER energy (Kcal/mol) | (-) CDOCKER Interaction energy (Kcal/mol) | No. of H bonds | Amino acid bound to H bond | Distance (Å) | Binding Energy (Kcal/mol) |
|---|---|---|---|---|---|---|---|---|
| Curry leaf phytocompounds | 1 | Histidinol | 26.2312 | 32.7445 | 3 | Trp372*<br>Glu377*<br>Arg199* | 1.80485<br>1.89176<br>2.2266 | -109.5131 |
| | 2 | Norpoproxyphene | 37.6774 | 47.5245 | 2 | Trp372*<br>Glu377* | 1.87396<br>1.97462 | -68.6032 |
| | 3 | Pheniramine | 26.8394 | 35.7931 | 1 | Trp372* | 1.93486 | -60.7209 |
| | 4 | Flucoxetine | 30.7307 | 40.6975 | 2 | Glu377*<br>Arg199* | 1.98375<br>2.42787 | -144.0215 |
| Standard antioxidant | 5 | BHA | 14.4034 | 22.6362 | - | - | - | - |
| Commercial drug | 6 | Apigenin | 35.1568 | 36.4487 | 1 | Tyr491 | 1.79155 | -63.421 |

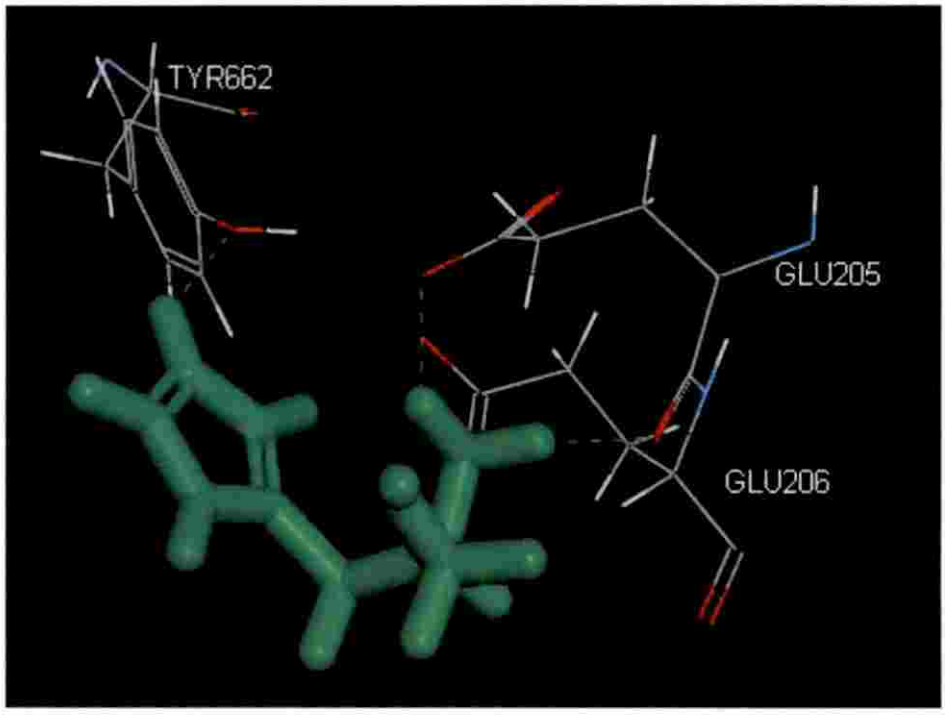



**Fig. 21: Hydrogen bond interaction of diabetes target dipeptidyl peptidase IV (1X70) with Histidinol**

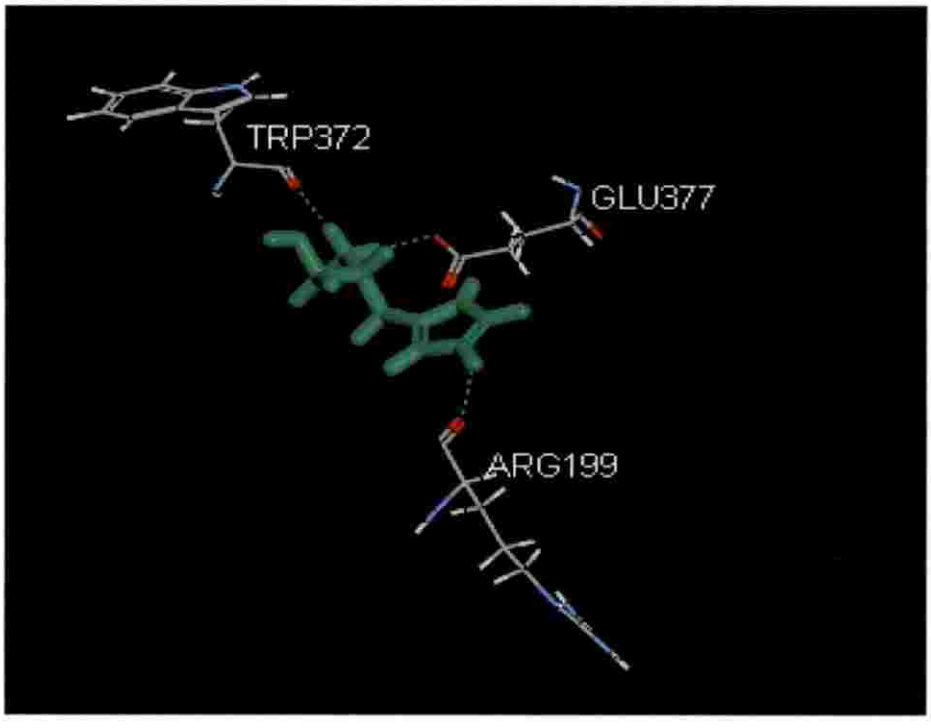



**Fig. 22: Hydrogen bond interaction of arthritis target nitric oxide synthase (4NOS) with Histidinol**

average binding energy of -63.621 kcal/mol but formed bond with noncritical amino acid. Interaction of BHA with target was unsuccessful as it failed to form any hydrogen bond with target.

#### *4.2.4.3.2 Cyclooxygenase-2 (COX-2)*

During molecular docking, three compounds of curry leaf interacted with target (Table 40). Compound valylmethionine recorded best docking scores with least deviation in between CDOCKER energy and CDOCKER interaction energy (0.9385 kcal/mol) and binding energy of -87.5187 kcal/mol. The interacting active residues was Arg120 made single hydrogen bond of 2.00236 Å (Fig. 23). Though, alpha-aminodiphenylacetic acid has good binding energy (-92.7008 kcal/mol), it interacted with noncritical amino acid. All compounds interacted to amino acid by forming single hydrogen bond.

BHA showed average interaction with target. The binding energy of BHA was -19.6085 kcal/mol and it interacted with target via active amino acid (Leu352) with formation hydrogen bond (2.48883Å) while commercial drug for target failed to produce any pose during docking.

#### *4.2.4.3.4 Vascular endothelial growth factor receptor (VEGFR)*

Result of molecular docking for vascular endothelial growth factor receptor with curry leaf phytocompounds is presented in Table 41. Valylmethionine recorded good coking score with -90.9075 kcal/mol binding energy. The difference between CDOCKER energy and CDOCKER interaction energy recorded less (2.2463 kcal/mol). The interacting amino acid reside was Asp1040 (Fig. 24). The length of hydrogen bond formed was 2.2463 Å. Histidinol interacted via two active residues of binding site.

Regorafenib, a commercial drug against vascular endothelial growth factor interacted via two active amino acid residues and binding energy of -46.4166 kcal/mol, but difference between CDOKCER energies crossed maximum difference (10 kcal/mol) required for stable binding. On other hand, binding of BHA to target was stable (-50.6318 kcal/mol binding energy and two hydrogen bonds).

#### *4.2.4.3.5 Glucocorticoid receptor (GR)*

Total four compounds (two from curry leaf, one FDA approved drug and BHA) interacted with Glucocorticoid receptor during molecular docking (Table 42). Histidinol recorded best docking score among all with less deviation between

**Table 40: Docking scores for the arthritis target cyclooxygenase-2 with selected ligands**

| Type of compound | Sl. No. | Ligand | (-) CDOCKER energy (Kcal/mol) | (-) CDOCKER Interaction energy (Kcal/mol) | No. of H bonds | Amino acid bound to H bond | Distance (Å) | Binding Energy (Kcal/mol) |
|---|---|---|---|---|---|---|---|---|
| Curry leaf phytocompounds | 1 | Valylmethionine | 44.3515 | 45.2900 | 1 | Arg120* | 2.00326 | -87.5187 |
| | 2 | Alpha-aminodiphenylacetic acid | 15.6369 | 20.0200 | 1 | Lys485 | 2.3160 | -92.7008 |
| | 3 | Histidinol | 22.2862 | 23.4808 | 1 | Met522* | 1.76121 | -16.5211 |
| Standard antioxidant | 4 | BHA | 21.7700 | 30.3743 | 1 | Leu352* | 2.48883 | -19.6085 |
| Commercial drug | 5 | Diclofenac | - | - | - | - | - | - |

**Table 41: Docking scores for the arthritis target vascular endothelial growth factor with selected ligands**

| Type of compound | Sl. No. | Ligand | (-) CDOCKER energy (Kcal/mol) | (-) CDOCKER Interaction energy (Kcal/mol) | No. of H bonds | Amino acid bound to H bond | Distance (Å) | Binding Energy (Kcal/mol) |
|---|---|---|---|---|---|---|---|---|
| Curry leaf phytocompounds | 1 | Histidinol | 25.2059 | 34.8500 | 2 | Glu878*(2) | 1.94055 | -122.0874 |
| | 2 | Valylmethionine | 37.0837 | 39.3300 | 1 | Asp1040* | 2.49925 | -90.9057 |
| Standard antioxidant | 3 | BHA | 26.4034 | 36.0700 | 2 | Lys861*<br>Val907* | 2.39229<br>1.97475 | -50.6318 |
| Commercial drug | 4 | Regorafenib | 36.8289 | 62.26 | 2 | Glu878*<br>Asp1040* | 1.97729<br>2.38382 | -46.4166 |

**Table 42: Docking scores for the arthritis target glucocorticoid receptor with selected ligands**

| Type of compound | Sl. No. | Ligand | (-) CDOCKER energy (Kcal/mol) | (-) CDOCKER Interaction energy (Kcal/mol) | No. of H bonds | Amino acid bound to H bond | Distance (Å) | Binding Energy (Kcal/mol) |
|---|---|---|---|---|---|---|---|---|
| Curry leaf phytocompounds | 1 | Histidinol | 24.831 | 29.2341 | 2 | Lys564*<br>Gln642* | 2.1305<br>2.0001 | -60.3297 |
| | 2 | Prometon | 33.5942 | 35.6988 | 1 | Leu563* | 2.22135 | -16.4472 |
| Standard antioxidant | 3 | BHA | 21.8181 | 29.7104 | 1 | Gln570* | 1.88481 | -25.5564 |
| Commercial drug | 4 | Dexamethasone | 0.748131 | 64.7806 | 3 | Asn564*<br>Arg611*<br>Gln642* | 2.16777 | -76.3907 |

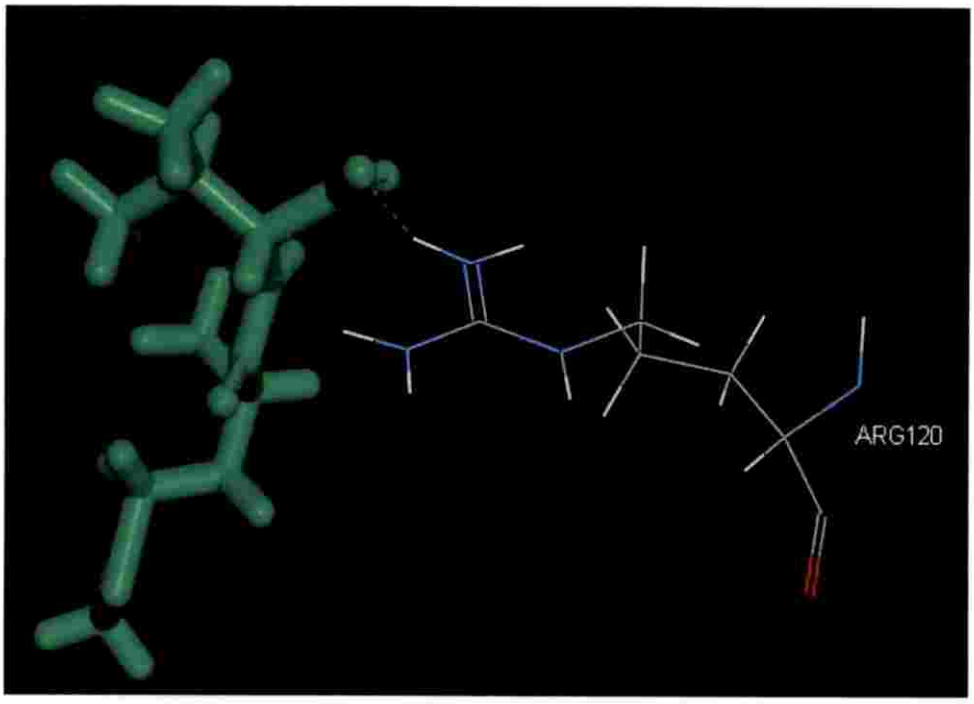



**Fig. 23: Hydrogen bond interaction of arthritis target cyclooxygenase (4COX) with Histidinol**

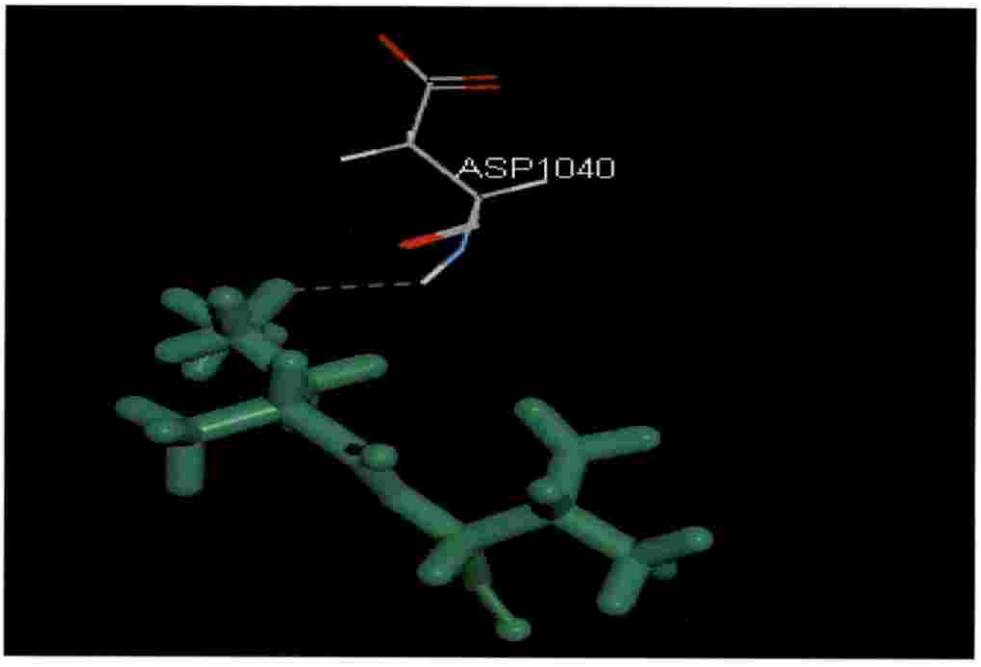



**Fig. 24: Hydrogen bond interaction of arthritis target vascular endothelial growth factor (3HNG) with Valylmethionine**

CDOCKCER energy and CDOCKER interaction energy (4.4041 kcal/mol) and biding energy of -60.3297 kcal/mol. The amino acids involved in interaction were Lys564, Gln642 (Fig.25). Prometon showed least deviation between CDOCKCER energy and CDOCKER interaction energy (2.1046 kcal/mol) but recorded low binding energy (-16.4472 kcal/mol).

Dexamethasone, commercial drug interacted with target (GR) but showed big deviation between CDOCKCER energy and CDOCKER interaction energy (64.0324 kcal/mol). BHA recorded average docking score with -25.5564 kcal/mol.

#### *4.2.4.4 Docking result of proteins (targets) associated with Alzheimer's*

##### *4.2.4.4.1 Human beta-secretase (BACE)*

While molecular docking with human beta-secretase, it was observed that three compounds from curry leaf interacted with target (Table 43). Number of hydrogen bonds formed between ligand and target was high for Histidinol (6) followed by two for valylmethonine and one for alpha-aminodiphenylacetic acid.

The difference between CDOKCER energy and CDOCKER interaction energy was least in valylmethonine (0.0468 kcal/mol). The binding energy recorded was the highest for Histidinol (-238.2687 kcal/mol). The shortest hydrogen bond length was recorded in interaction of valylmethonine with target (amino acid Gly291; distance 1.9279 Å) while largest was observed in interaction of Histidinol with target via Gln134 with 2.45039 Å (Fig. 26). Histidinol recorded the best docking score. The inhibitors of beta-secratase are under clinical trials while standard antioxidant, BHA failed to generate any pose during docking.

##### *4.2.4.4.2 Tau protein kinase 1*

With tau protein kinase 1, three compounds interacted in docking process (Table 44). The alpha-aminodiphenylacetic acid recorded best docking score. The CDOCKER energy and CDOCKER interaction energy was least for Histidinol (1.8645 kcal/mol). Alpha-aminodiphenylacetic acid recorded the highest binding energy of -108.29 kcal/mol followed by flucoxetine and histidinol. The interacting amino acid residues was Ile62 (Fig. 27). Histidinol scored good docking score with -63.8241 kcal/mol binding energy and six hydrogen bonds with lowest length (1.85452 Å) for hydrogen bond amongst all, formed via Asp200.

**Table 43: Docking scores for the Alzheimer's target human beta-secratase 1 with selected ligands**

| Type of compound | Sl. No. | Ligand | (-) CDOCKER energy (Kcal/mol) | (-) CDOCKER Interaction energy (Kcal/mol) | No. of H bonds | Amino acid bound to H bond | Distance (Å) | Binding Energy (Kcal/mol) |
|---|---|---|---|---|---|---|---|---|
| Curry leaf phytocompounds | 1 | Histidinol | 43.1859 | 49.6054 | 6 | Thr292*<br>Asp93(3)*<br>Gly291*<br>Gln134* | 2.04077<br>2.10050<br>2.05078<br>2.45039 | -238.2687 |
| | 2 | Alpha-aminodiphenylacetic acid | 20.6784 | 28.0667 | 1 | Gln134* | 2.03195 | -59.5881 |
| | 3 | Valylmethionine | 41.5066 | 41.5534 | 3 | Thr293(2)*<br>Gly291* | 2.11557<br>1.9279 | -42.6927 |
| Standard antioxidant | 4 | BHA | - | - | - | - | - | - |

**Table 44: Docking scores for the Alzheimer's target tau protein kinase with selected ligands**

| Type of compound | Sl. No. | Ligand | (-) CDOCKER energy (Kcal/mol) | (-) CDOCKER Interaction energy (Kcal/mol) | No. of H bonds | Amino acid bound to H bond | Distance (Å) | Binding Energy (Kcal/mol) |
|---|---|---|---|---|---|---|---|---|
| Curry leaf phytocompounds | 1 | Alpha-aminodiphenylacetic acid | 31.5291 | 36.7163 | 1 | Ile62*<br>Arg141* | 2.25997<br>1.90876 | -108.2900 |
| | 2 | Histidinol | 27.1869 | 29.0514 | 6 | Asp200(3)*<br>Asn186*<br>Lys85*<br>Asn64* | 1.85452<br>2.08457<br>2.04984<br>2.42771 | -63.8241 |
| | 3 | Flucoxetine | 26.0445 | 35.5866 | 4 | Val135*<br>Asn186(2)*<br>Asp200* | 2.22949 | -75.8159 |
| Standard antioxidant | 4 | BHA | 16.9391 | 24.8146 | 1 | Val135* | 2.3692 | -48.7135 |
| Commercial drug | 5 | Verbecestat | 11.3293 | 50.3069 | 1 | Gln134(2)* | 2.017 | -127.3836 |

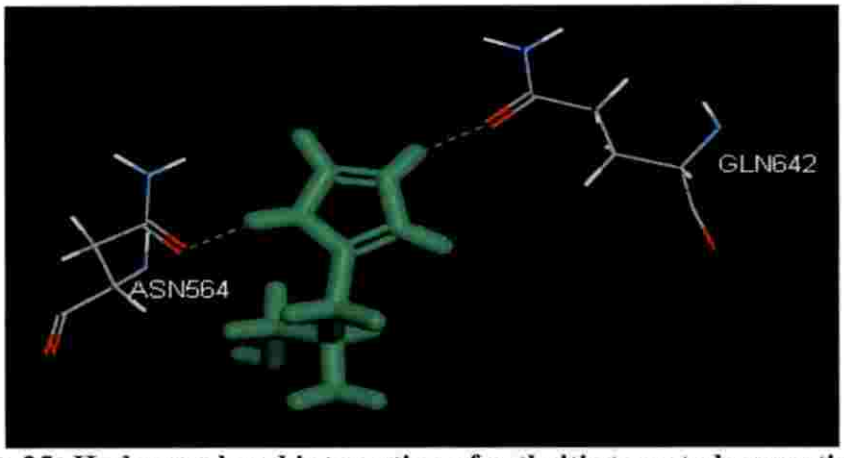



**Fig. 25: Hydrogen bond interaction of arthritis target glucocorticoid receptor (1M2Z) with Histidinol**

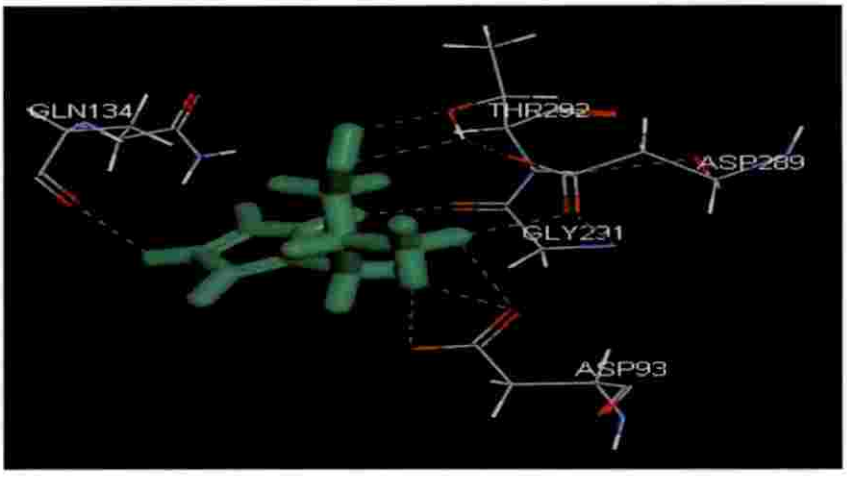



**Fig. 26: Hydrogen bond interaction of Alzheimer's target human beta secratse 1 (2XFJ) with**

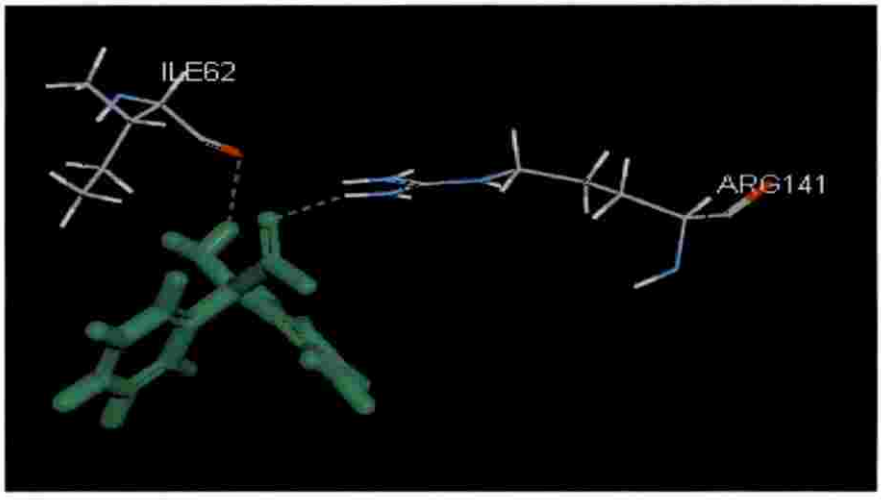



**Fig. 27: Hydrogen bond interaction of Alzheimer's target tau protein (1J1B) with Alpha-aminodiphenylacetic acid**

The commercial drug verbecestat interacted with target through active amino acid Gln134 by forming hydrogen bond of 2.017 Å with high binding energy (-127.3836 kcal/mol), but the difference between CDOCKER energy and CDOCKER interaction energy was higher, surpassed the maximum limit. The BHA interacted with acceptable docking score.

#### *4.2.4.4.3 Human acetylcholinesterase (AChE)*

During docking of human acetylcholinesterase with curry leaf compound and commercial drugs, four compounds (two from curry leaf, one drug and one standard antioxidant) interacted with target (Table 45). Amongst all, Histidinol recorded high binding energy with target (-146.6949 kcal/mol) but formed hydrogen bond via noncritical amino acid in target. Valylmethionine recorded best docking score for the target with least deviation in between CDOKCER energies (0.108 kcal/mol) and binding energy of -12.6644 kcal/mol. It interacted with target (AChE) via two active amino acid (Tyr337 and His447) (Fig. 28).

The FDA approved drug, galanthamine interacted to active amino acid Tyr337 with -42.2004 kcal/mol binding energies but CDOCKER energy was positive while BHA interacted with acceptable docking score.

#### *4.2.4.4.4 Human butyrylcholinesterase (BChE)*

Molecular docking results of human butyrylcholinesterase with curry leaf compounds and commercial drug is presented in Table 46. Valylmethonine scored best docking score while interacting with target. The binding energy recorded for this interaction was -70.7351 kcal/mol. The difference between CDOCKER energy and CDOCKER interaction energy was least (0.1632 kcal/mol). It interacted to target via three critical amino acids viz. Gln116, Gly117 and His438 (Fig. 29). The length of hydrogen bonds formed during this interaction was 2.27936, 2.16967 and 2.23236 Å respectively.

The highest binding energy was observed during interaction of alpha-aminodiphenylacetic acid to target (-79.9481 kcal/mol) followed by valylmethonine and histidinol while number of hydrogen bonds formed was highest in valylmethonine (6) trailed by Histidinol and alpha-aminodiphenylacetic acid (2).

The commercial drug, rivastigmine interacted with butyrylcholinesterase but failed to form hydrogen bonds while in case of BHA no pose generated during docking.

**Table 45: Docking scores for the Alzheimer's target human acetylcholineestrease with selected ligands**

| Type of compound | Sl. No. | Ligand | (-) CDOCKER energy (Kcal/mol) | (-) CDOCKER Interaction energy (Kcal/mol) | No. of H bonds | Amino acid bound to H bond | Distance (Å) | Binding Energy (Kcal/mol) |
|---|---|---|---|---|---|---|---|---|
| Curry leaf phytocompounds | 1 | Valylmethionine | 30.8723 | 30.9803 | 3 | Tyr337*<br>His447(2)* | 2.24963<br>1.92247 | -12.6644 |
| | 2 | Histidinol | 31.104 | 36.9254 | 2 | Tyr341<br>Asp74 | 2.2288<br>1.97653 | -146.6949 |
| Standard antioxidant | 3 | BHA | 21.0624 | 29.4544 | 1 | His447* | 1.94325 | -26.2499 |
| Commercial drug | 4 | Galanthamine | -4.14846 | 43.6394 | 1 | Tyr337* | 2.05222 | -42.2004 |

**Table 46: Docking scores for the Alzheimer's target human butyrylcholinesterase with selected ligands**

| Type of compound | Sl. No. | Ligand | (-) CDOCKER energy (Kcal/mol) | (-) CDOCKER Interaction energy (Kcal/mol) | No. of H bonds | Amino acid bound to H bond | Distance (Å) | Binding Energy (Kcal/mol) |
|---|---|---|---|---|---|---|---|---|
| Curry leaf phytocompounds | 1 | Histidinol | 20.0987 | 24.5631 | 2 | Leu286*<br>Pro285 | 1.79638<br>1.96302 | -57.6284 |
| | 2 | Alpha-aminodiphenylacetic acid | 26.8401 | 34.8137 | 2 | His438*<br>Gly117* | 2.3415<br>2.16595 | -79.9481 |
| | 3 | Valylmethionine | 34.9731 | 35.1363 | 4 | Gln116*<br>Gly117(2)*<br>His438* | 2.27936<br>2.16967<br>2.23236 | -70.7351 |
| Standard antioxidant | 4 | BHA | - | - | - | - | - | - |
| Commercial drug | 5 | Rivastigmine | 23.3692 | 49.257 | | - | - | - |

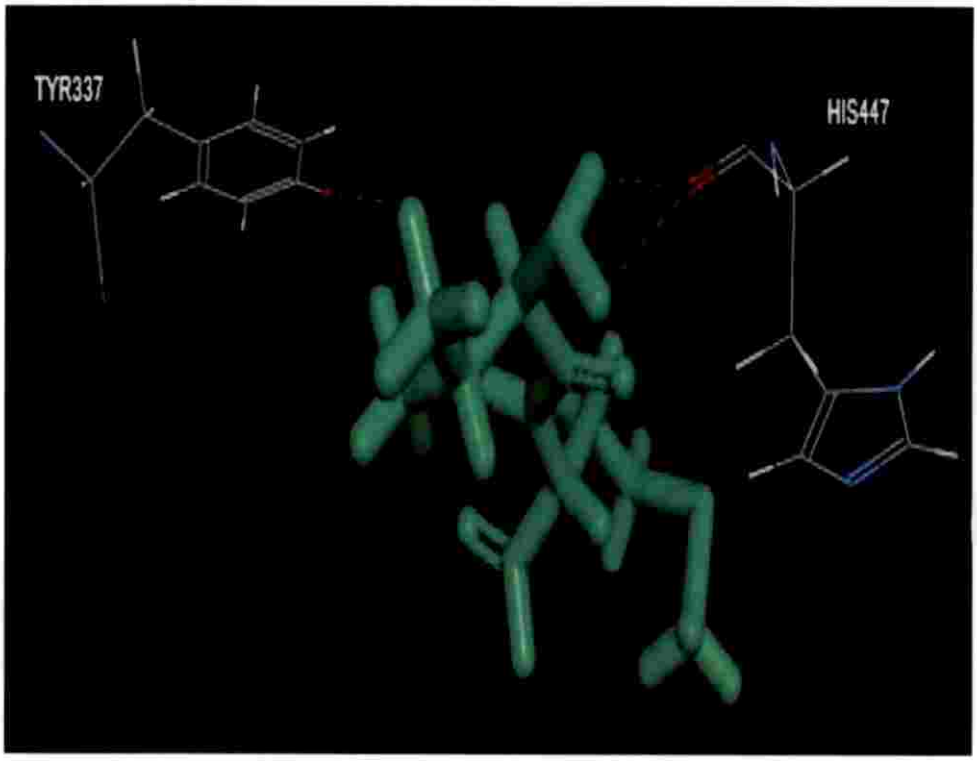



**Fig. 28: Hydrogen bond interaction of Alzheimer's target human acetylcholinesterase with (4BDT) Valylmethionine**

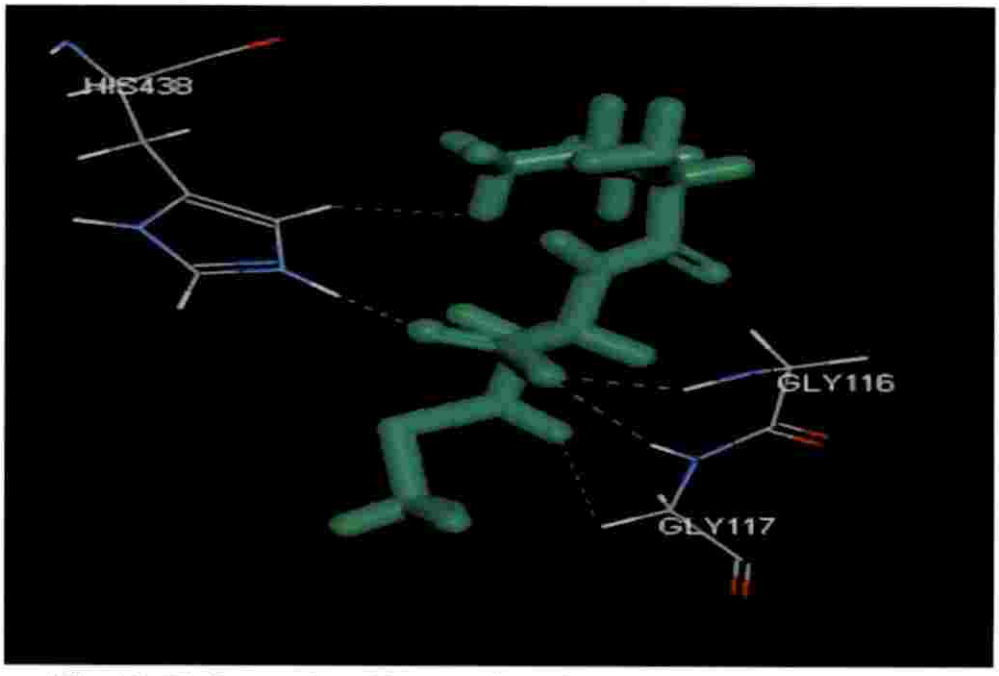



**Fig. 29: Hydrogen bond interaction of Alzheimer's target human butyrylcholinesterase (4BDS) with Valylmethionine**

### 4.2.5 ADME/T Prediction

The pharmacokinetic properties of ligands and approved drugs were analysed. It includes Solubility, Human Intestinal Absorption, Blood Brain-Barrier (BBB) penetration, hepatotoxicity and Cytochrome P450 inhibition level. ADME/T analysis was performed using ADME/T descriptor protocol of Discovery Studio 4.0. Descriptor levels of the analogs obtained from the same (Table 3). The result of ADMET analysis is presented in Table 47 and Table 48. Solubility level varied between 2 to 5 for curry leaf ligands. flucoxetine and norpropoxyphene showed low solubility (2) while histidinol found to be too soluble (5). The solubility level varied from1 to 5 for approved drugs. regorafenib, tamoxifen, fulvestrant and fenretinide found to be extremely low while lithium carbonate was too soluble. Absorption level for curry leaf ligand ranged from 0 to 1. Histidinol showed moderate absorption level while all other ligands were shown good level of absorption. In case of approved drugs absorption level ranged from 0 to 3. Absorption level was good for apigenin, diclofenac, dicholoroacetic acid, diphenylamine and epalrestat while fulvestrant, NSC-54776 and Technetium tc99m medronate showed poor level of absorption.

Blood Brain-barrier level varied between 0-4 for curry leaf ligands. Flucoxetine was found to be highly penetrative while for Histidinol it is very poor. Among commercial drugs, tamoxifen was highly penetrative while BBB level was very poor for fenretinide, fulvestrant, NSC-54776, lithium carbonate and regorafenib. Hepatotoxic prediction proved to be false for all curry leaf ligands except prometon while among commercial drugs, dexamethasone, epalrestat, fulvestrant, galanthamine and trimetrexate found to be safe, rest all were toxic. The CYP2D6 prediction was found to be true for three curry leaf ligand i.e. doxylamine, flucoxenine and norpropoxyphene while it was false for rest all. In case of approved drugs only diclofenac was found to be true while it was false for rest all.

### 4.2.6 Summary of results from molecular docking analysis

Interaction of nine compounds from curry leaf with different targets for cancer, diabetes, arthritis and Alzheimer's were good as per the analysis of docking results (Table 49). Among the phytocompounds, histidinol interacted with highest number of targets. It showed interaction with seven targets for cancer, six targets for diabetes, four targets each for arthritis and Alzheimer's. Valylmethionine, a dipeptide was second highest in terms of interaction with targets. It interacted with three targets for cancer,

**Table 47: ADME/T properties of ligands from curry leaf interacted against cancer, diabetes, arthritis and Alzheimer's**

| Sl No. | Compound name | Pubchem I.D. | ADMET Solubility level | ADMET Absorption level | ADMET BBB level | Hepatotoxic Prediction | CYP2D6 Prediction |
|---|---|---|---|---|---|---|---|
| 1 | Alpha-aminodiphenylacetic acid | 18289 | 3 | 0 | 3 | FALSE | FALSE |
| 2 | DL-2-aminooctanoic acid | 69522 | 4 | 0 | 3 | FALSE | FALSE |
| 3 | Doxylamine | 3162 | 3 | 0 | 1 | FALSE | TRUE |
| 4 | Flucoxetine | 3386 | 2 | 0 | 0 | FALSE | TRUE |
| 5 | Histidinol | 776 | 5 | 1 | 4 | FALSE | FALSE |
| 6 | Norpropoxyphene | 18804 | 2 | 0 | 1 | FALSE | TRUE |
| 7 | Pheniramine | 4761 | 3 | 0 | 1 | FALSE | FALSE |
| 8 | Prometon | 4928 | 3 | 0 | 2 | TRUE | FALSE |
| 9 | Valylmethionine | 292427 | 4 | 0 | 3 | FALSE | FALSE |

**Table 48: ADMET properties of commercial approved drugs for cancer, diabetes, arthritis and Alzheimer's**

| Sl. No. | Compound name | PubChem I.D. | ADMET Solubility level | ADMET Absorption level | ADMET BBB level | Hepatotoxic Prediction | CYP2D6 Prediction |
|---|---|---|---|---|---|---|---|
| 1 | Apigenin | 5280443 | 3 | 0 | 3 | TRUE | FALSE |
| 2 | Dexamethasone | 5743 | 3 | 0 | 3 | FALSE | FALSE |
| 3 | Diclofenac | 3033 | 2 | 0 | 1 | TRUE | TRUE |
| 4 | Dicloroacetic acid | 6597 | 4 | 0 | 2 | TRUE | FALSE |
| 5 | Diphenylamine | 11487 | 3 | 0 | 1 | TRUE | FALSE |
| 6 | Epalrestat | 1549120 | 2 | 0 | 2 | FALSE | FALSE |
| 7 | Fenretinide | 5288209 | 1 | 1 | 4 | TRUE | FALSE |
| 8 | Fulvestrant | 17756771 | 1 | 3 | 4 | FALSE | FALSE |
| 9 | Galanthamine | 9651 | 3 | 0 | 2 | FALSE | FALSE |
| 10 | Lithium carbonate | 11125 | 5 | 2 | 4 | TRUE | FALSE |
| 11 | Myricetin | 5281672 | 3 | 3 | 4 | TRUE | FALSE |
| 12 | Nsc-54776 | 104758 | 3 | 3 | 4 | TRUE | FALSE |
| 13 | Regorafenib | 11167602 | 1 | 0 | 4 | TRUE | FALSE |
| 14 | Ribociclib | 44631912 | 2 | 0 | 3 | TRUE | FALSE |
| 15 | Rivastigmine | 77991 | 3 | 0 | 1 | TRUE | FALSE |
| 16 | Tamoxifen | 2733526 | 1 | 1 | 0 | TRUE | FALSE |
| 17 | Technetium tc99m medronate | 131704315 | 5 | 3 | 4 | TRUE | FALSE |
| 18 | Trimetrexate | 5583 | 2 | 0 | 4 | FALSE | FALSE |
| 19 | Verubecestat | 51352361 | 2 | 0 | 4 | TRUE | FALSE |

**Table 49: Details of curry leaf phytocompounds interaction with targets of cancer, diabetes, arthritis and Alzheimer's**

| Sl. No. | Compound name | No. of targets interacted | | | |
|---|---|---|---|---|---|
| | | Cancer | Diabetes | Arthritis | Alzheimer's |
| 1 | Alpha-aminodiphenylacetic acid | 4 | 4 | 1 | 3 |
| 2 | DL-2-aminooctanoic acid | - | 1 | - | - |
| 3 | Doxylamine | 1 | 1 | - | - |
| 4 | Flucoxetine | 2 | 2 | 1 | 1 |
| 5 | Histidinol | 7 | 6 | 4 | 4 |
| 6 | Norpropoxyphene | - | - | 1 | 1 |
| 7 | Pheniramine | 3 | 2 | 1 | 0 |
| 8 | Prometon | 1 | 1 | - | - |
| 9 | Valylmethionine | 3 | 6 | 2 | 3 |

six targets for diabetes, two targets for arthritis and three targets for Alzheimer's. DL-2 aminooctanic acid interacted with single target for diabetes.

Strong inhibition of target is related with number of compound interacting with target at critical amino acid residue in active site of target. Analysis of molecular docking results of curry leaf phytocompounds with different targets for cancer, diabetes, arthritis and Alzheimer's revealed variation in inhibition (Table 50). For cancer, maximum number of compounds interacted with breast cancer proteins 17 β-HSD and polo-loke kinase 1. On other hand only one compound of curry leaf interacted with targets like dihydrofolate reductase, human androgen receptor estrogen receptor ligand binding domain. Out of seven targets for diabetes, maximum compounds from curry leaf interacted with fructose 1,6-bisposhpatase and human dipeptidyl peptidase IV while single compound from compound interacted with target human aldose reductase. With arthritis target nitric oxide synthase maximum number of curry leaf compounds showed interaction. Out of four target for Alzheimer's, maximum number of curry leaf compounds interacted with human beta-secretase 1, tau protein kinase and human butyrylcholinesterase while single compound interacted with acetylcholinesterase
Though, Histidinol was good in terms of interaction and it was observed that solubility was too less. Valylmethionine recorded good values for ADME/T analysis. Alpha-aminodiphenylacetic acid was another compound to score good values in ADME/T analysis. So three compounds *viz.* alpha-aminodiphenylacetic acid, DL-2-aminooctanoic acid and valylmethionine showed a good interaction with different targets for cancer, diabetes, arthritis and Alzheimer's which infers the inhibitory action of these compounds on wide range of diseases, passed criteria of ADME/T analysis which infers the safety of compound for human consumption can be tested further on cell lines or model animals to ensure its activity on live systems.

**Table 50: Details of interaction of target proteins with number of curry leaf phytocompounds**

| Sl. No. | Disease | | Target proteins | No. of phytocompounds interacted | |
|---|---|---|---|---|---|
| | | | | Critical residue | Non-critical residue |
| 1 | Cancer | 1 | 17 beta-HSD (Breast Cancer) | 4 | 0 |
| | | 2 | Polo-like kinase 1 (Breast Cancer) | 4 | 0 |
| | | 3 | Exchange protein directly activated by CAMP (Pancreatic cancer) | 2 | 1 |
| | | 4 | Nat -2 receptor (All type of cancer) | 3 | 0 |
| | | 5 | Phosphoinositide-3 kinase (All type of cancer) | 2 | 1 |
| | | 6 | Human androgen receptor (Prostate Cancer) | 1 | 0 |
| | | 7 | Dihydropholate reductase (Colon cancer) | 1 | 0 |
| | | 8 | Human estrogen receptor ligand-binding domain (Breast Cancer) | 1 | 0 |
| 2. | Diabetes | 1 | Fructose-1, 6-bisphosphatase | 4 | 0 |
| | | 2 | Human glucokinase | 3 | 0 |
| | | 3 | Glycogen synthase kinase | 3 | 1 |
| | | 4 | Pyruvate dehydrogenase kinase | 2 | 2 |
| | | 5 | Human aldose reductase | 1 | 1 |
| | | 6 | Human multidrug resistance protein | 2 | 0 |
| | | 7 | Human dipeptidyl peptidase IV | 4 | 1 |
| 3. | Arthritis | 1 | Nitric oxide synthase | 4 | 0 |
| | | 2 | Cyclooxygenase-2 | 2 | 1 |
| | | 3 | Vascular endothelial growth factor receptor | 2 | 0 |
| | | 4 | Glucocorticoid receptor | 2 | 0 |
| 4. | Alzheimer's | 1 | Human beta Secratase 1 | 3 | 0 |
| | | 2 | Tau protein kinase 1 | 3 | 0 |
| | | 3 | Human acetylcholineestrease | 1 | 1 |
| | | 4 | Human butyrylcholinesterase | 3 | 0 |

# *Discussion*

# 5. Discussion

The results of the study entitled as 'Characterization of antioxidant fractions in curry leaf (*Murraya koenigii* L.) and molecular docking of selected bioactive compounds' are discussed under in this chapter.

## 5.1 EVALUATION OF ANTIOXIDANT FRACTIONS FROM CURRY LEAF

### 5.1.1 Extraction of oleoresin

Recovery of oleoresin extracted from mature curry leaf was higher (9.16%) than oleoresin extracted from medium mature leaves (7.87%). Oleoresin comprises of complete flavor and non-volatile resinous fraction present in the spice. The resinous fraction consists of pigments, fixative, a natural antioxidant, and heat components (Sharma and Sharma, 2012). Accumulation of chemicals in oleoresin is reported to increase with maturity (Mathai, 1981). Apart from these, the method of oleoresin extraction, as well as the difference of variety, has an influence over variation in accumulation of various chemicals (Ghasemzadeh *et al.*, 2014; Gahlot *et al.*, 2018). This may be the reason behind the higher recovery of oleoresin of mature curry leaf.

### 5.1.2 Antioxidant assay

Oleoresin extracted from mature leaves showed higher per cent inhibition (85.19%) than oleoresin extracted from medium mature leaves (83.30%). The oleoresin extracted using acetone possessed a high quantity of total phenols (Salomi and Manimekalai, 2016).

The maturity of leaves has a direct influence on the total content of phenols and mature leaves are reported to contain a high concentration of phenols (Anwar *et al.*, 2017). Several researchers have explained positive correlation between increasing phenolic content and antioxidant activity (Jacobo-Velázquez and Cisneros-Zevallos, 2009; Albayaray *et al.*, 2010; Baba *et al.*, 2015, Adebiyia *et al.*, 2017).

### 5.1.3 Separation of antioxidant fractions from curry leaf by column chromatography

#### *5.1.3.1 Fractionation of oleoresin*

Oleoresin was fractionated by silica gel column chromatography using solvent systems composed of hexane: ethyl acetate in different proportions *viz.* 100:0, 80:20, 60:40, 40:60, 20:80 and 0:100 per cent ethyl acetate. The yield of extract in fraction

60:40 was higher (707.4 mg). The selection of solvent systems is based on sample used. Initial elution with 100 per cent hexane yielded less as compared to fraction 60:40. This can be attributed to interaction between hexane and silica gel. Silica gel has $SiO_2$ and oxygen atoms dangling at surface of silica binds to protons. Presence of hydroxyl ions which makes whole column polar. To elute compounds on stationary phase (Silica), solvent has to compete with analyte for binding to surface of silica. Hexane is a nonpolar solvent and hence cannot replace analytes from surface of silica. During elution with Hexane, compounds loosely bound to silica gel moves out of column along with solvent while polar solvent (Ethyl acetate) strongly compete with analyte and replaces it. The analyte moves along with solvent. Yield of fraction extracted using 100 per cent ethyl acetate was less among all because most of analyte was eluted during extraction with 60:40 (hexane: ethyl acetate) and 40:60 (hexane: ethyl acetate). Rao *et al.* (2007) isolated bioactive compounds (carbazole alkaloids) from curry leaf using silica gel column chromatography and elution of analyte was done using Hexane: ethyl acetate. Bajapai *et al.* (2011) isolated and characterized sugiol (an antibacterial compound) from *Metasequoia glyptostroboides* using chromatography with Hexane: ethyl acetate as an eluting solvent.

#### *5.1.3.2 Antioxidant assay*

Radical scavenging activity of each fraction was determined. Inhibition of free radical was higher in fraction (60:40 hexane: ethyl acetate) followed by fraction (40:60 hexane: ethyl acetate), fraction (80:20 hexane: ethyl acetate), fraction (20:80 hexane: ethyl acetate), fraction (100:0 hexane: ethyl acetate) and fraction (0:100 hexane: ethyl acetate). Standard antioxidant butylated hydroxianisole recorded the highest per cent of free radical inhibition (Olubami *et al.*, 2014).

Antioxidant potential of plants are evaluated by several methods like 2,2'-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging method, static headspace gas chromatography (HS-GC), β-carotene bleaching test (BCBT), Ferric ion Reducing Antioxidant Power assay (FRAP), Thiobarbituric acid reactive substance (TBARS), [2,2'-azinobis(3-ethylbenzothiazoline-6-sulphonic acid)] free radical scavenging activity assay (ABTS), etc. (Gupta, 2015). Each assay has advantages and disadvantages. Static headspace gas chromatography (HS-GC) method is highly sensitive but requires sophisticated instruments while β-carotene bleaching test (BCBT) does not require sophisticated instruments but affected by the polarity of

sample (Koleva *et al.*, 2002). A great disadvantage with FRAP method is a time required for chemical preparation while for ABTS method radicals have to generate before assay (Shah and Medi, 2015). Among all DPPH is very rapid, simple, sensitive, reproducible and does not require special instrumentation. Also alcoholic DPPH solution is used as a TLC spray reagent for rapid detection of radical scavengers before spectrophotometric measurements (Takao *et al.*, 1994; Koleva *et al.*, 2002 ). Hence DPPH is a widely preferred method for evaluation of antioxidant potential of plant extract (Ramsevak *et al.*, 1999; Tachibana *et al.*, 2002; Rao *et al.*, 2007; Nigappa *et al.*, 2008).

### *5.1.4 Sub-fractionation of Fraction (60:40 hexane: ethyl acetate) and antioxidant assay*

Sub-fractionation of fraction (60:40 hexane: ethyl acetate) at five minute interval was done using column chromatography. Total 47 sub-fractions were collected and subjected to antioxidant assay. The 34$^{th}$ fraction recorded higher inhibition of free radicals followed by 26$^{th}$, 28$^{th}$, 38$^{th,}$ and 40$^{th}$. The inhibition per cent of sub-fractions were more than the fraction (60:40 hexane: ethyl acetate). This may be due to the presence of large number of compounds in fraction (60:40 hexane: ethyl acetate). Bendary *et al.* (2013) reported that antiradical activity, Hydrogen peroxide scavenging activity and DPPH inhibition activity of phenol was less compared to individual phenolic compound catechol. Individual compound isolated from curry leaf oleoresin showed higher inhibition of DPPH than total oleoresin (Rao *et al.*, 2007). A standard antioxidant BHA, recorded the highest inhibition of free radicals (91.89%). The inhibition per cent of 26$^{th}$, 28$^{th}$ and 34$^{th}$ sub-fraction was 91.08, 91.51 and 91.08 per cent respectively which were on par with BHA. Hence, it can be stated that sub-fractions (26$^{th}$, 28$^{th,}$ and 34$^{th}$) showed an almost similar potential as that of BHA in terms of DPPH inhibition. Thus, this sub-fractions can be used as alternative to BHA and without any side effects which are associated with use of BHA (Hocman, 1988).

### *5.1.5 LC-MS/MS analysis*

LC-MS/MS analysis was done for one whole fraction (60:40 hexane: ethyl acetate) and five sub-fractions viz. 26$^{th}$, 28$^{th}$, 34$^{th}$, 38$^{th,}$ and 40$^{th}$. From each fraction 100 compounds were analysed and a total 98 different compounds were identified. Medicinal roles of some of compounds identified by LC-MS/MS analysis are tabulated in Table 51.

**Table 51: Medicinal properties of compounds identified through LC-MS/MS analysis from different fractions of curry leaf**

| Sl. No. | Compound Name | Medicinal properties |
|---|---|---|
| 1 | 17-Epistanozolol | Used in angioedema |
| 2 | 18-Hydroxycorticosterone | Acts as Na and K exchanger |
| 3 | 24-Nor-9,11-seco-11-acetoxy- 3,6-dihydroxycholest-7,22- dien-9-one | Anticancer agent |
| 4 | 26,27-diethyl-1alpha,25- dihydroxy-22-thiavitamin D3 / 26,27-diethyl-1alpha,25-dihydroxy-22-thiachole | Anticanrcer agent |
| 5 | Ansamitocin P3 | Antibiotic and antitumor agent |
| 6 | Cinnarizine | Antihsitamine and antiallergic agent |
| 7 | Cucurbitacin D | Anticancer (Cervical cancer) |
| 8 | Cucurbitacin I | Antidiabetic |
| 9 | Cucurbitacin L | Antidiabetic |
| 10 | Demethylcitalopram | Antidepressant |
| 11 | Dopexamine | Increases blood flow (used in heart surgery) |
| 12 | doxylamine | Antihistamine agent |
| 13 | Droperidol | Antinausea agent |
| 14 | Erythromycin C | Macrolide antibiotic |
| 15 | Fluoxetine | Antidepressant |
| 16 | Indoprofen | Anti-inflammatory drug |
| 17 | Ipecac (Emetamine) | Anti-protozoal |
| 18 | Lorazepam | Antiolytic |
| 19 | Monensin | Antibiotic |
| 20 | Moricizine sulfoxide | Antirrhymatic agent |
| 21 | Morphinone | Analgesic agent |
| 22 | Octocrylene | Cosmetic (Sunburn protection) |
| 23 | Ouabain | Antirrhymatic drug |
| 24 | Oxybutynin | Antispasmodics agent |

| | | |
|---|---|---|
| 25 | Pheniramine | Antihistamine |
| 26 | Probucol spiroquinone | Anticholosteolemic |
| 27 | Propafenone | Antirrhymatic agent |
| 28 | Saquinavir | Protease inhibitor |
| 29 | Vitamin D3 glucosiduronate | Treatment of rickets |

Compounds in spice crops and medicinal plants are identified using different techniques like high performance liquid chromatography (HPLC), gas chromatography-mass spectrometry (GCMS), nuclear magnetic resonance (NMR), liquid chromatography- Mass spectrometry (LC-MS) and LC-MS/MS. Compound identification using HPLC is done by comparing retention time for standard peak with sample peak requires a standard compound. If standard is not available identification becomes difficult also different compounds can have the same retention time which may result in biased findings. The GC-MS is widely applied for volatile compound identification. The sample preparation often problem with GC-MS to make difficult compound amenable for analysis. It includes liquid-liquid extraction (LLE), solid phase extraction (SPE), and/or derivatization. Sample preparation for LC-MS and LC-MS/MS is simple and does not need derivatization. Though sample preparation includes solid-phase extraction (SPE) or liquid-liquid extraction (LLE) it is as simple as direct injection, dilution or precipitation (www.absiex.com). In LC-MS during compound identification, only precursor ion is analysed while when MS/MS is used first MS filters precursor ion does fragmentation while second filters product of fragmentation which increases sensitivity of analysis. Tribalat *et al.* (2006) reported the high selectivity of LC-MS/MS over LC-MS for detection nitrofuran parent drug in honey (Tribalat *et al.*, 2006). Hence, LC-MS/MS analysis widely used by several researchers for identification of compounds in different spices and crops (Keskes *et al.*, 2017; Bakari *et al.*, 2018; Choi *et al.*, 2018; Pawar and Nasreen, 2018).

### 5.2 Identification of bioactive compounds by *in silico* analysis

In the present study, the interaction of 69 curry leaf phytocompounds with eight targets for cancer, seven targets for diabetes, four targets each for arthritis and Alzheimer's was assessed using 'Discovery studio v. 4.0' software. Ligands were filtered using Lipinski rule (Lipinski, 2004) and out of 79 ligands, 43 ligands passed rule while 42 ligands failed to pass the rule. Out of seven drugs for cancer one drug,

Fulvestrant failed to pass Lipinski rule while all six drugs for diabetes, four drugs for arthritis and three drugs for Alzheimer's passed the Lipinski rule.

The target protein analysed by 'Discovery studio v. 4.0' for finding out the active site and critical amino acid in the active sites. These critical amino acids interacted with ligands by forming hydrogen bond and ensured inhibition of target protein activity to cure disease.

## 5.3 MOLECULAR DOCKING AND ANALYSES

Molecular docking was performed using 'CDCOKER' protocol of 'Discovery studio v. 4.0'. Selection of active inhibitors for lifestyle diseases like cancer, diabetes, arthritis and Alzheimer's was based on low binding energy (Ramakanth *et al.*, 2012). The selection of ligand was also based on the deviation between CDOCKER energy and CDOCKER interaction energy. The energy of internal ligand, as well as receptor energy, forms CDKOCER energy while CDKOCKER interaction energy is the energy between protein and ligand and based on the values, the binding between target and ligand was estimated. Lower the deviation, stronger the interaction. The difference of more than ten between CDKOCER energy and CDKOCKER interaction energy for ligand and target protein interaction was unstable and hence neglected (Chilom *et al.*, 2006).

### 5.3.1 Interaction of curry leaf phytocompounds with cancer target

With the caner target 17-beta HSD, Valylmethionine from curry leaf showed good interaction with minimum deviation in between CDOCKER energy and CDOCKER interaction energy (0.0388 kcal/mol) and with good binding energy (-66.7903 kcal/mol). Valylmethionine interacted at active site residues of target 17-beta HSD and inhibited the target. Hence, Valylmethionine can be used as a drug candidate to inhibit the target 17-beta HSD which modulates breast cancer protein and impacts cell migration (Aka *et al.*, 2012) as well as reported for increase in pheochromocytoma cell growth and resistance to cell death due to overexpression (Carlosn *et al.*, 2015). Commercial drug Fulvestrant failed to pass Lipinski rule which shows its poor bioavailability as an oral drug.

With the target Polo-like kinase 1, valylmethionine, pheniramine showed good interaction. Valylmethionine recorded the least deviation between CDOCKER energy and CDOCKER interaction energy and interacted at critical amino acid (Lys 82) of

active site of PLK-1. Interaction of commercial drug with the target was unstable due to as value for CDOKER energy was positive. Interaction of Valylmethionine with amino acid critical for activity of polo-like kinase 1 result in inhibition of polo-like kinase 1 which is involved several cancer like Non-small-cell lung cancer (Wolf *et al.*, 2000), head and neck cancer (Knetch *et al.*, 2000), Esophageal and gastric cancer (Tokumistu *et al.*, 1999), Melanomas (Strebhardt *et al.*, 2000; Kneisel *et al.*, 2002), ovarian cancer (Takai *et al.*, 2001; Weichert *et al.*, 2004), colorectal cancer (Macmillan *et al.*, 2001) and thyroid cancer (Ito *et al.*, 2004).

Almahariq *et al.* (2012) reported that a novel compound 3-(5-tert-butyl-isoxazol-3-yl)-2-[(3-chlorophenyl)-hydrazono]-3-oxo-propionitrile (ESI-09) inhibited pancreatic cell growth and migration by interacting with EPAC2 via tertbutyl-isoxazolyl moiety at Phe367, Leu406, Ala407 residues and tert-butyl-isoxazolyl moiety at Glu 404. Similarly, curry leaf phytocompounds like alpha-aminodiphenylacetic acid and valylmethionine interacted with EPAC2 at Glu404 residue while commercial drug diphenylamine failed to form any hydrogen bond with the target. The Epac proteins are involved in several type of cancers like lung cancer, melanoma, prostate cancer, lymphatic leukemia, brain cancer and ovarian cancer (Kumar *et al.*, 2017).

The alpha-aminodiphenylacetic acid, pheniramine, and histidinol interacted with NAT-2 protein. Alpha- aminodiphenylacetic acid recorded least difference between CDOCKER energy and CDOCKER interaction energy and formed three hydrogen bonds with Ser216, amino acid of the active site. This interaction may cause inhibition of target NAT-2 protein. Commercial drug NSC-5476, also showed good interaction but formed only one hydrogen bond with target NAT-2. The target NAT-2 is involved in many of types of cancer *viz.* Lung cancer (Seow *et al.*, 1999), colorectal cancer (Windmill *et al.*, 2000), urinary prostate cancer (Hamasaki *et al.*, 2003) bladder cancer (Wu *et al.*, 2016).

With target phosphoinositide-3 phosphate (PI3K), three compounds from curry leaf interacted. Histidinol recorded the highest binding energy. Walker *et al.* (2000) studied the structural determinants of phosphoinositide 3-kinase inhibition by wortmannin, LY294002, quercetin, myricetin, and staurosporine and observed that inhibitors are binding in pocket made up of residue 831, 879, 880 and 881 and side pocket consist of 950,953,961,963,964. Histidinol and doxylamine interacted with target protein via Glu880 and Met 953 suggests that binding these compounds inhibits PI3K.

Only a single compound interacted with human androgen receptor (AR). Histidinol showed high binding energy and interacted with critical amino acids (Thr877, Asn705, and Leu704) in the binding site of AR. Commercial drug fenrtinide showed positive CDOCKER energy while nilutamide interacted with active amino acid residue but showed large deviation difference between CDOCKER energy and CDOCKER interaction energy. Binding of Histidinol results may result in inhibition of human androgen receptor which is widely used target for treatment of prostate cancer (Crawford *et al.*, 2018; Einstein *et al.*, 2019) as well as breast cancer (Giovannelli *et al.*, 2018; Vasiliou *et al.*, 2019).

The phytocompound histidinol from curry leaf interacted with target dihydrofolate reductase with good docking energy and less deviation between CDOCKER energy and CDOCKER interaction energy. Commercial drug trimetrexate, interacted with the highest docking energy but recorded high deviation in CDOCKER energy and CDOCKER interaction energy. Histidinol interacted with target DHFR at amino acid Ile5 and Asp27 while commercial drug trimextrexate interacted at Ile5, Asp27, and Ile94 with more binding energy than Histidinol. Similarly, Al-Harabi *et al.* (2015) reported inhibition of DHFR with synthesized Schiff bases. Schiff bases interacted with target at different amino acid residue viz. ILe 5, Ala 6, Ala7, Asp 27, Leu 28, Phe 31, Lys 32, Ser 49, Ile 50, Arg 52, Leu 54, Arg 57, Ile 94, Tyr 100.

With estrogen receptor ligand binding domain, only histidinol interacted with high docking energy. It formed a hydrogen bond with Leu346 and Glu353.Commercial drug trimextrexate interacted with Asp351 with binding energy less than histidinol. Brzozowski *et al.* (1997) reported antagonist action of raloxifen and agonist action ostradiol on estrogen receptor and found that raloxifen interacted with Glu353, Asp351, Leu391, Arg394, Leu 402, His524 and Leu525. It shows that histidinol can be used to target estrogen receptor ligand binding domain.

### 5.3.2 Interaction of curry leaf phytocompounds with diabetes target

With target Fructose 1, 6-bisphosphatase, four curry leaf compounds showed interaction. Valylmethionine recorded the least deviation between CDOCKER energy and CDOCKER interaction energy with good binding energy. All ligands interacted with Glu26 and Thr31 amino acid in the active site of target (FBpase). Binding of ligands at Glu26, Thr31 of FBpase increases potency of bound compound to inhibit the enzyme (FBpase) (Hibsen *et al.*, 2008; Mahendrana *et al.*, 2014)

Three compounds from curry leaf interacted with human glucokinase. DL-2 aminooctanoic acid recorded higher docking energy as well as the least deviation between CDOCKER energy and CDOCKER interaction energy. It interacted with amino acid (Lys169, Gly229) critical for the functioning of glukokinase which may lead to inhibition of glucokinase. Inhibition of glucokinase is considered a promising strategy to control diabetes as overexpression of glucokinase caused reduction in binding of glucose to glucokinase leads to impaired insulin secretion (Bell *et al.*, 1999).

With target glycogen synthase kinase-3 (GSK-3) four curry leaf compounds interacted. Valylmethionine showed good interaction with target protein with less difference in CDOCKER energy and CDOCKER interaction energy. Among four, two compound viz. valylmethionine and histidinol and commercial drug lithium carbonate interacted with amino acid Val135 in the active site of while two compounds *viz.* valylmethionine and flucoxetine interacted with amino acid Tyr 135. Curcumin reported to interact with the same residue of GSK-3 and resulted in inhibition of GSK-3 (Bustanji *et al.*, 2009).

Molecular docking of pyruvate dehydrogenase kinase (PDK) showed the interaction of four curry leaf compounds. Alpha-aminodiphenylacetic acid and flucoxetine interacted with active amino acids of PDK. Valylmethionine interacted with Arg258, an amino acid not predicted as active by Discovery Studio. But with Arg258 of PDK (4Z, 12Z)-cyclopentadeca-4, 12-dienone (a novel compound from *Grewia hirsute*) formed a strong hydrogen bond and blocked the activity of PDK (Natrajan *et al.*, 2015).

Human aldose reductase interacted with two compounds of curry leaf *viz.* alpha-aminodiphenylacetic acid and valylmethionine with good docking scores. Alpha-aminodiphenylacetic acid as well as commercial drug, epalrestat interacted with active amino acid residue Ser210 and Gly262 respectively with high binding energy. Binding of compounds at these residues (Ser210 and Gly262) leads to inhibition of target human aldose reductase which is reported to involve in the pathogenesis of many diseases *viz.* inflammatory diseases, ovarian diseases, cancer, renal insufficiency and ischemic condition (Sangshetti *et al.*, 2014).

With a target human multidrug resistance protein (MDRP), curry leaf compound histidinol and valylmethionine, as well as commercial drugs Polaprezinc and Technetium Tc-99m medronate, showed interaction with active amino acid of MDRP. The interacting amino acids were Gln713 and Lys684. These active amino acids are

critical for function of MDRP as predicted by Discovery studio 4.0. Binding phytocompounds as well as commercial drugs to active amino acid residue may inhibit the Multidrug resistance protein which is major cause of chemotherapy resistant cancer and targeted to reverse the multidrug resistance (Dhasmana *et al.*, 2018).

Human dipeptidyl peptidase-IV (DPP IV) interacted with five compounds of curry leaf, a standard antioxidant, and two standard drugs. Histidinol showed the best interaction with DPP4. Curry leaf phytocompounds interacted with different active amino acids of DPP4 viz. Arg125, Glu205, Glu296, Tyr622 and Asn710. Kim *et al.* (2005) studied inhibition of DPP4 using novel inhibitor ((2R)-4-Oxo-4-[3-(Trifluoromethyl)-5,6-dihydro[1,2,4]triazolo[4,3-a]pyrazin-7(8H)-yl]-1-(2,4,5-trifluoro-phenyl) butan-2-amine) and it was observed that the inhibitor interacted with same amino acids residue mentioned above which indicated a strong inhibition of DPP4.

### 5.3.3 Interaction of curry leaf phytocompounds with arthritis target

With target nitric acid synthase (NOS), four curry leaf compounds interacted. Histidinol recorded good docking score with high binding energy and less deviation between CDOCKER energy and CDOCKER interaction energy. All compounds formed a hydrogen bond with Arg199, Trp372, Glu377 and Tyr491 present in an active site. Inhibitors of NOS generally interacted with Glu377 and leads to strong inhibition of NOS (Fischmann *et al.*, 1999).

Cyclooxygenase is an important enzyme in prostaglandin synthesis. Level of COX reported higher during inflammation (Gately, 2000). The COX is associated with several diseases such as cancer, rheumatoid arthritis (Sonwane *et al.*, 2011). Curry leaf phytocompounds interacted with amino acids present in active site. Valylmethionine showed good docking score as well as binding energy. It interacted with Arg120 of COX-2. Similar kind of inhibition was recorded for COX-2 (Kurumbail *et al.*, 1996).

Vascular endothelial growth factor (VEGFR) interacted with two compounds of curry leaf. Valylmethionine recorded good docking score. Compounds interacted with active amino residues in (Glu878, Lys861, Val907, Asp1040) binding sites of VEGR which can cause inhibition of target VEGR which is associated with several kind of disease condition such as neurological disorders (Lange *et al.*, 2016), eye disease (Penn *et al.*, 2008) and cancer (Lackal *et al.*, 2018).

With the target Glucocorticoid receptor, histidinol and prometon showed interaction. Among them, histidinol showed high binding energy and interacted at

active amino acids viz. Leu 563, Lys564, Gln642, Gln570 and Arg611. Commercial drugs, Dexamethasone also interacted strongly with the target Glucocorticoid receptors. Binding at active site amino acids may cause Glucocorticoid inhibition which is reported to cause inflammation and risk of cardiovascular diseases (Kadmiel and Cidlowski, 2013).

### 5.3.4 Interaction of curry leaf phytocompounds with Alzheimer's target

Human beta-secretase I is an important target to treat Alzheimer's disease. Inhibition of beta-secretase occurs when the protein conformation is changed due to binding of inhibitor at the catalytic site. Catalytic sites for BACE1 Alzheimer's are Thr 292, Asp 93, Asp 289, Thr 293, Gln 134, Asn294, and Thr 133 (Atlam *et al.*, 2018). Curry leaf compounds such histidinol, alpha-aminodiphenylacetic acid and valylmethionine interacted with BACE1 target at Thr292, Asp93, Gln291, and Gln134 which indicated the potential of curry leaf phytocompounds for inhibition of BACE-1.

With Tau protein kinase four phytocompounds of curry leaf, standard antioxidant and FDA approved drug showed interaction. Tau protein is inhibited by drugs during treatment of Alzheimer's. Inhibition occurs when inhibitors bind to the catalytic site of Tau protein composed Of Lys 85, Asp 133, Tyr 134, Val 135, Asp 200 (Barai *et al.*, 2018). Flucoxetine and Histidinol interacted with Tau protein at Asp200, Lys85, Asn64, Val135, ASn186, Gln134. It showed that curry leaf compounds have the potential to inhibit Tau protein.

Human acetylcholinesterase (AchE) is one of the promising targets for Alzheimer's treatment. AChE breaks the neurotransmitter signals and converts them into acetic acid and choline. Larik *et al.* (2018) reported that the inhibition of AchE at particular amino acids viz. Trp86 and Tyr337 cause a reduction in activity of protein. Among the curry leaf phytocompounds, valylmethionine interacted with Tyr337 and His447 amino acids of active site proteins which infers the significant inhibition of target protein. Approved drug galanthamine also showed interaction with critical amino acid Tyr337.

Human butyrylcholinesterase (BchE) interacted with three phytocompounds of curry leaf. Valylmethionine interacted with good docking score by forming three hydrogen bonds at Gln116, Gly117 and His438. These amino acids are predicted as active by Discovery studio thus binding of compounds to this amino acids causes inhibition of BchE. Apart from target for Alzheimer's, BchE is used as a biomarker in

Parkinson's disease (Dong *et al.*, 2017) and higher activity of BchE increases risk of coronary artery diseases (Alcantara *et al.,* 2002).

## 5.4 ADME/T ANALYSIS OF LIGANDS

After molecular docking, compounds were analysed using ADME/T to check bioavailability of compounds inside the human body. Several drugs are taken back from market due to toxicity issues and hence screening of drugs by ADME/T is widely preferred (Valerio 2009; Butina *et al.*, 2002). Five different parameters solubility level, absorption level, blood brain barrier level, hepatotoxic prediction and CYP2D6 prediction were analysed using 'ADME/T descriptor' tool of Discovery studio v. 4.0. All these parameters were analysed using mathematical calculations (Reddy *et al.*, 2012). All ligands and commercial drugs were categorized into three groups based on values of parameters viz. Acceptable (A), highly acceptable (HA) and Non acceptable (NA). The highly acceptable group will include compounds satisfying all acceptable limits of parameters. Acceptable group contains compounds which are not in the acceptable range for two parameters while non-acceptable group contains compound not in acceptable range for three or more parameters.

Among nine curry leaf ligands, only three compounds were found as highly acceptable. These compounds (alpha-aminodiphenylacetic acid, DL-2 aminooctanoic acid and valylmethionine) were found easily absorbed in body, easily soluble in body fluids, non-inhibitor in action on CYP2D6 drug metabolizing enzyme with less penetration in CNS and exhibited non-toxic effect on liver.

Remaining six compounds (doxylamine, flucoxetine, histidnol, norpropoxyphene, pheniramine and prometon) were found as acceptable. These compounds were either too soluble like Histidinol or interference with CYP2D6 enzymes like doxylamine and nopropoxyphene. Some compounds like doxylamine, nopropoxyphene, and pheniramine were high penetrant in the central nervous system while Prometon showed hepatotoxicity.

Among 19 commercial drugs, only four *viz.* dexamethasone, galanthamine, epalrestat and trimetrexate were found as highly acceptable while the remaining 14 were found acceptable. One drug *viz.* diclofenac was found non-acceptable due to high penetration in CNS, toxicity to liver and high interference with CYP2D6 enzyme.

Histidinol interacted with 21 target out of 23 target studied. It was found as one of the promising phytocompound from curry leaf but was too soluble as per ADME/T

analysis. Solubility of drug can be enhanced by physical and chemical methods (Chaudhary *et al.*, 2012). Hence, efforts can be made to improve solubility of histidinol.

The overall result from *in vitro* assay and molecular docking studies confirmed the antioxidant potential of curry leaf and could pin point bioactive compounds responsible for inhibition of targets for diseases like cancer, diabetes, arthritis and Alzheimer's. Curry leaf sub-fractions ($26^{th}$, $28^{th}$ and $34^{th}$) recorded DPPH inhibition similar to synthetic antioxidant BHA which confirms the high antioxidant potential of curry leaf. Three potential bioactive compounds *viz.* alpha-aminodiphenylacetic acid, DL-2 aminooctanoic acid and valylmethionine were found as safe and can be advanced as potential drug candidates. Among three, Valylmethionine is a dipeptide for which no pharmaceutical activity has been reported yet but possessed good docking score and found highly acceptable in ADME/T analysis. Validation of these compounds using cell lines and animal models will further ensure the protective actions of these compounds in living systems and can be further advanced for drug development.

The study could bring about the potential of curry leaf as a natural antioxidant and could identify three safe phytocompounds *viz.* alpha-aminodiphenylacetic acid, DL-2-aminooctanoic acid and valylmethionine which could interact with targets for cancer, diabetes, arthritis and Alzheimer's.

# *Summary*

# 6. Summary

The study entitled "Characterization of antioxidant fractions in curry leaf (*Murraya koenigii* L.) and molecular docking of selected bioactive compounds" was carried out at the Centre for Plant Biotechnology and Molecular Biology (CPBMB) and Distributed Information Centre (DIC), College of Horticulture, Kerala Agricultural University, during 2017-2019. The objectives of the study were to characterize antioxidant fractions in curry leaf through in vitro assays and to identify the most potent bioactive compound through LC-MS/MS and molecular docking analyses.

The salient findings of the study are summarised in this chapter.

**Separation of antioxidant fractions from curry leaf by column chromatography**

- High recovery of oleoresin (9.16 %) was recorded in mature leaves of curry leaf.
- Oleoresin extracted from mature leaves recorded higher inhibition of DPPH activity (85.19 %).
- Fraction (60:40 hexane: Ethyl acetate) recorded highest yield of extract (707.4 mg).
- Higher DPPH inhibition (88.68%) was recorded in fraction extracted using Hexane: Ethyl acetate in proportion of 60:40
- Subfractionation of fraction (60:40 Hexane: Ethyl acetate) at five minute interval yielded 47 subfractions. Subfraction $34^{th}$ recorded higher inhibition of DPPH (91.51%) followed by $26^{th}$ (91.08%), $28^{th}$ (91.08), $38^{th}$ (89.53%) and $40^{th}$ (89.53%).
- LC-MS/MS analysis of whole fraction (60:40 Hexane: Ethyl acetate) and five subfractions ($26^{th}$, $28^{th}$, $34^{th}$, $38^{th}$, and $40^{th}$) identified 100 compounds from each sample.
- The maximum number of compounds were identified in $26^{th}$ subfraction (62) followed by the whole fraction 60:40 Hexane: Ethyl acetate (52), $28^{th}$ subfraction (51), $34^{th}$ subfraction (51), $38^{th}$ subfraction (49), and $40^{th}$ subfractions (45).
- In whole fraction (60:40 Hexane: Ethyl acetate), 73.07 per cent of compounds were with peak area from 1147776 to 5756137.
- In $26^{th}$ sub-fraction, 40.32 per cent of compounds were with peak area ranging from 4828291 to 2495209.

- In 28th sub-fraction, 64.70 per cent of compounds were with peak area ranging from 7854246 to 3981987.
- In 34th sub-fraction, 66.66 per cent of compounds were with peak area ranging from 5347165.8 to 2737972.4.
- In 38th sub-fractions, 55.10 per cent of compounds were with peak area ranging from 8589818.4 to 4371156.2
- In 40th sub-fraction, 62.22 per cent of compounds were with peak area ranging from 6573810 to 13114124.
- Norpropoxyphene was the most abundant compound in whole fraction (60:40 Hexane: Ethyl acetate), 28th subfraction and 38th subfraction.
- In 26th, sub-fraction, Norprochlorperazine was found as the most abundant compound.
- In 34th sub-fraction, Gibberellin A36 was found as the most abundant compound.
- In 40th subfraction, Phrobol was found as the most abundant compound.
- The 26th subfraction possessed the maximum number of unique compounds (13) followed by 40th subfraction (10), whole fraction [60:40 Hexane: Ethyl acetate] (6), 28th (5), 38th (3) and 34th (2).
- Curry leaf subfractions (26th, 28th and 34th) recorded DPPH inhibition similar to synthetic antioxidant BHA which confirmed the high antioxidant potential of curry leaf.

**Molecular docking and analysis**

- Among 79 ligands of curry leaf, 32 failed to pass the Lipinski rule.
- Among 19 commercially approved drugs, one drug Fulvestrant failed to pass the Lipinski rule.
- Seven compounds of curry leaf (Alpha-aminodiphenylacetic acid, Doxylamine, Flucoxetine, Histidinol, Pheniramine, Prometon and valylmethionine) found to interact with different targets for cancer. Maximum inhibition was observed for targets 17 beta-HSD (Breast Cancer), Polo-like kinase 1 (Breast Cancer). Histidinol interacted with seven out of eight targets studied for cancer while Alpha-aminodiphenylacetic acid interacted with four targets.
- With seven different targets studied for diabetes, eight phytocompounds (Alpha-aminodiphenylacetic acid, DL-2-aminooctanoic acid, Doxylamine, Flucoxetine, Histidinol, Pheniramine, Prometon and Valylmethionine) showed interaction.

Histidinol and Valylmethionine interacted with six out of seven targets studied for diabetes. Maximum inhibition was observed for target human dipeptidyl peptidase IV and fructose1,6 bisphosphatase.

- Six compounds of curry leaf (Alpha-aminodiphenylacetic acid, Flucoxetine, Norpropoxyphene, Histidinol, Pheniramine and Valylmethionine) were interacted with four different targets of arthritis studied. Maximum inhibition was observed for target Nitric oxide synthase. Histidinol interacted with all the four targets studied for arthritis.
- Five phytocompounds (Alpha-aminodiphenylacetic acid, Flucoxetine, Norpropoxyphene, Histidinol and valylmethionine) showed interaction with different targets for Alzheimer's. Histidinol interacted with all the four targets while Alpha-aminodiphenylacetic acid and Valylmethionine interacted with three targets each. Among the four targets for Alzheimer's, less inhibition was observed for target human acetylcholineesterase.
- Among the interacted phytocompounds of curry leaf, Alpha-aminodiphenylacetic, DL-2 aminooctanoic acid and Valylmethionine were found as highly acceptable compounds as per ADME/T analysis.
- Six compounds (Doxylamine, Flucoxetine, Histidinol, Norpropoxypehene, Pheniramine, Prometon) found to be in the category of acceptable.
- Among 19 commercial drugs docked, four (Dexamethasone, Epalrestat, Galanthamine and Trimetrexate) were observed as highly acceptable while one drug Diclofenac was considered as non-acceptable.
- Molecular docking and ADME/T analysis revealed the potential of three (Alpha-aminodiphenylacetic, DL-2 aminooctanoic acid and valylmethionine) phytocompounds as a safe and potent inhibitor of targets for cancer, diabetes, arthritis and Alzheimer's.
- Valylmethionine is a novel compound not yet reported for its medicinal properties but possessed good docking score and found highly acceptable in ADME/T analysis.

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16

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182

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# Annexures

# Annexures I

## List of chemical and other items used for *In vitro* study

| Sl. No. | Product Name | Company |
|---|---|---|
| 1 | Butylated hydroxyanisole | Sigma Aldrich |
| 2 | Hexane | SRL |
| 3 | Ethyl acetate | SRL |
| 4 | Silica gel (200-400 ) | SRL |
| 5 | Methanol | SRL |
| 6 | 2,2-diphenyl-1-picrylhydrazyl | SRL |
| 7 | Glass column | Nice equipment |
| 8 | Plastic Cuvettes | Tarson |

# Annexure II

## List of laboratory equipment/software's used for study

| Sl. No. | Items | Procured from |
|---|---|---|
| 1 | Discovery Studio 4.0 | Accelrys, USA |
| 2 | Laminar air flow | Labline industries, India |
| 3 | Spectrophotometer | Genesys (Thermo Scientific) |
| 4 | Centrifuge | Hitachi Japan |
| 5 | Water bath | Rotek |
| 6 | Soxhlet apparatus (Heating mantle) | Rotek |

# Abstract

# CHARACTERIZATION OF ANTIOXIDANT FRACTIONS IN CURRY LEAF (*Murraya koenigii* L.) AND MOLECULAR DOCKING OF SELECTED BIOACTIVE COMPOUNDS

By

**Bhamare Deepak Prashant**

**(2017-11-001)**

## ABSTRACT OF THE THESIS

**Submitted in partial fulfillment of the requirement for the degree of**

## MASTER OF SCIENCE IN AGRICULTURE

**(PLANT BIOTECHNOLOGY)**

**Faculty of Agriculture**

**Kerala Agricultural University, Thrissur**


**CENTRE FOR PLANT BIOTECHNOLOGY AND MOLECULAR BIOLOGY**

**COLLEGE OF HORTICULTURE**

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193

## Abstract

Curry leaf (*Murraya koenigii* L.) belonging to the family Rutaceae is one of the extensively used spices in traditional Indian medicine against variety of ailments. Curry leaf is reported to possess several pharmaceutical properties such as antioxidant, anticancerous, antidiabetic, anti-inflammatory, antidiarrheal, analgesic and hepatoprotective. The therapeutic potential of curry leaf is due to several chemical constituents such as carbazole alkaloids, phenols, flavonols, tannins, terpenes, and lipids.

The overproduction of reactive oxygen species (ROS) serve as the initiation point for many diseases like cancer, diabetes, arthritis and Alzheimer's. The ROS can be scavenged by antioxidants but side effects have been reported for synthetic antioxidants like butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT). Hence, natural antioxidants are gaining scientific attention now-a-days. Though, the pharmacological potential of curry leaf is well understood, very few reports are available on the role of bioactive phytocompounds for curing diseases by interacting with target proteins.

The study entitled "Characterization of antioxidant fractions in curry leaf (*Murraya koenigii* L.) and molecular docking of selected bioactive compounds" was undertaken with the objective to characterize antioxidant fractions in curry leaf through *in vitro* assays and to identify the most potent bioactive compound through LC-MS/MS and molecular docking analyses.

Oleoresin was extracted from curry leaf (var. Suvasini) and further subjected to *in vitro* antioxidant assay. Antioxidant fractions from curry leaf were separated by silica gel column chromatography using hexane: ethyl acetate solvent system in various proportions (100:0, 80:20, 60:40, 40:60, 20:80 and 0:100) and further subjected to antioxidant assay. Sub-fractionation of the fraction exhibiting the highest antioxidant activity was done at five minutes interval and sub-fractions were also subjected to antioxidant assay. Sub-fractions exhibiting maximum antioxidant activity were analyzed by LC-MS/MS. Compounds identified through LC-MS/MS analysis were docked against eight target proteins for cancer, seven for diabetes, four for arthritis and four for Alzheimer's.

Mature leaves of curry leaf recorded higher oleoresin recovery of 9.16 per cent and possessed high antioxidant activity with a DPPH inhibition of 85.19 per cent. Fraction eluted with hexane: ethyl acetate (60:40) recorded the highest yield of extract (707.4 mg) and showed the highest antioxidant activity with 88.68 per cent inhibition of

DPPH. Sub-fractionation of the fraction with the highest antioxidant activity has yielded 47 sub-fractions. Of the sub-fractions, 28th fraction showed the highest DPPH inhibition (91.51%) followed by 26th (91.08%), 34th (91.08), 38th (89.53%) and 40th (89.53%). The DPPH inhibition potential of sub-fractions 28th, 26th and 34th was similar to synthetic antioxidant BHA (91.89%). The LC-MS/MS analysis of these fractions revealed presence of 52 compounds in whole fraction (hexane: ethyl acetate 60:40), 62 in 26th sub-fraction, 51 in 28th and 34th sub-fraction, 49 in 38th sub-fraction and 45 in 40th sub-fraction.

Seven compounds of curry leaf *viz.* alpha-aminodiphenylacetic acid, doxylamine, flucoxetine, histidinol, pheniramine, prometon and valylmethionine were found to interact with different targets for cancer. Maximum number of curry leaf phytocompounds interacted with targets for breast cancer, 17β hydroxysteroid dehydrogenase (17β HSD) and Polo-like kinase 1 (PLK1). valylmethionine inhibited 17β HSD and PLK1 with good binding energy of -66.7903 and -122.5233 kcal/mol respectively.

Eight phytocompounds of curry leaf *viz.* alpha-aminodiphenylacetic acid, DL-2-aminooctanoic acid, doxylamine, flucoxetine, histidinol, pheniramine, prometon and valylmethionine interacted with seven different targets for diabetes. Maximum number of compounds interacted with the target fructose 1,6-bisphosphatase and valylmethionine inhibited the target with good docking score with a binding energy of -81.143 kcal/mol.

Six compounds of curry leaf *viz.* alpha-aminodiphenylacetic acid, flucoxetine, norpropoxyphene, histidinol, pheniramine and valylmethionine interacted with four different targets of arthritis studied. Maximum number of compounds interacted with the target Nitric oxide synthase and it was inhibited by histidinol with good binding energy -109.5131 kcal/mol.

Five phytocompounds *viz.* alpha-aminodiphenylacetic acid, flucoxetine, norpropoxyphene, histidinol and valylmethionine interacted with four different targets for Alzheimer's. Maximum number of compounds interacted with targets human beta-secretase 1, tau protein kinase and human butyrylcholinesterase. Good docking score was recorded for interaction of human beta-secretase 1 with histidinol.

The study could bring about the potential of curry leaf as a natural antioxidant and could identify three safe phytocompounds *viz.* alpha-aminodiphenylacetic acid, DL-2-aminooctanoic acid and valylmethionine which could interact with targets for cancer, diabetes, arthritis and Alzheimer's.