173727

MOLECULAR DOCKING AND VALIDATION OF MEDICINAL EFFECTS OF COCONUT (*Cocos nucifera* L.)

By

DEVI LEKSHMI S

(2013-11-192)

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

VELLANIKKARA, THRISSUR - 680 656

KERALA, INDIA

2016

DECLARATION

I hereby declare that the thesis entitled **"Molecular docking and validation of medicinal effects of coconut (*Cocos nucifera* L.)"** 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.

Vellanikkara
Date: 30-1-2016

Devi Lekshmi S
(2013-11-192)

CERTIFICATE

Certified that the thesis entitled **"Molecular docking and validation of medicinal effects of coconut (*Cocos nucifera* L.)"** is a bonafide record of research work done independently by **Mrs. Devi Lekshmi S (2013-11-192)** under my guidance and supervision and that it has not previously formed the basis for the award of any degree, diploma, associateship, fellowship to her.

Vellanikkara
Date: 30-1-2016

Dr. P. A. Nazeem
(Chairman, Advisory Committee)
Professor and Co-ordinator (DIC)
Centre for Plant Biotechnology
and Molecular Biology
College of Horticulture,
Vellanikkara, Thrissur

CERTIFICATE

We, the undersigned members of the advisory committee of **Mrs. Devi Lekshmi S (2013-11-192)**, a candidate for the degree of **Master of Science in Agriculture**, with major field in **Plant Biotechnology**, agree that the thesis entitled **"Molecular docking and validation of medicinal effects of coconut (*Cocos nucifera* L.)"** may be submitted by **Mrs. Devi Lekshmi S**, in partial fulfilment of the requirement for the degree.

Dr. P. A. Nazeem
(Chairman, Advisory Committee)
Professor and co-ordinator (DIC)
Centre for Plant Biotechnology and Molecular Biology
College of Horticulture,
Vellanikkara, Thrissur

29/01/2016

Dr. P. A. Valsala
(Member, Advisory committee)
Professor and Head
Centre for Plant Biotechnology
and Molecular Biology
College of Horticulture
Vellanikkara, Thrissur

Dr. M. R. Shylaja
(Member, Advisory committee)
Professor
Centre for Plant Biotechnology
and Molecular Biology
College of Horticulture
Vellanikkara, Thrissur

Dr. C. R. Achuthan
(Member, Advisory committee)
Assistant Professor
Dept. of Biotechnology
Amala Cancer Research Centre
Thrissur

Anantharamanan Rajamani
(External Examiner)
Scientist- Genome Lab
RRII

ACKNOWLEDGEMENT

I bow my head before the lotus feet of **Almighty**, for blessing me with health, strength and courage to get through all tedious circumstances and to complete this endeavor.

At this moment of accomplishment, my heart is overwhelmed with gratitude and I wish if these words could convey the subtle feelings.

With great respect and devotion, I would like to express the deepest gratitude to my major advisor **Dr. P. A. Nazeem**, Professor, CPBMB, College of Horticulture, Vellanikkara, for her valuable and inspiring guidance, esteemed advise, unfailing patience, friendly approach, constant support and encouragement throughout the research work and during the preparation of the thesis, without which fulfillment of this thesis would not have been possible. I really consider it my greatest fortune in having her guidance for my research work and my obligation to her lasts forever.

I wish to express my sincere gratitude to **Dr. P. A. Valsala**, Professor and Head, CPBMB, College of Horticulture, Vellanikkara, member of my advisory committee for the valuable guidance, timely suggestions and help rendered in the conduct of the experiment.

I am extremely thankful to **Dr. M. R. Shylaja**, Professor, CPBMB, College of Horticulture, Vellanikkara, member of my advisory committee for her support, suggestions and guidance throughout the study.

I also owe my deep sense of gratitude and heartfelt thanks to **Dr. C. R. Achuthan**, Assistant Professor, Dept. of Biotechnology, Amala Cancer Research Center, Thrissur, member of my advisory committee, for his valuable guidance and timely help rendered in the conduct of the experiment.

Words fall short as I place on record my indebtedness to **Dr. Abida P. S** and **Dr. Deepu Mathew** of CPBMB and **Dr. T. D Babu** of Amala Cancer Research Center for their untiring support and valuable guidance during my course of study.

I take this opportunity to express my heartfelt thanks to **Bincy, Sangeetha, Priyanga, Misha, Dhanasree, Priya, Meenu, Vipin** and **Ravi** of DIC, for their warm support and concern for my completion of thesis. I express my thanks to **Shailaja chechi** and **Simi chechi** who helped me in several ways for the completion of this venture.

I express my special thanks to **Arun, Jeksi, Indhu** and **all other members** of **Amala Cancer Research Center, Thrissur** for their technical support and guidelines for the successful completion of my thesis.

Words cannot really express the true friendship that I relished from my dear ones **Judy, Arfa, Sara, Deepthi, Kunjan, Mallik, Sanjeev and Abhi.** Their prayers, constant support and loving words of comfort helped me a lot in all the hardships. My heartfelt gratitude cannot be captured in words for the unflinching support, constant encouragement and warm concern of my friends **Roy, Irfana, Sree, Asha, Pallavi, Dola, Shelsa, Anakha, Manikesh, Shruti, Vinutha, Ramesh, Kalilu , Naresh and Manjalam** for the past two years. I thank **Aryechi, Gritz, Nimz and Rashu,** whose prayers and sincere love rendered me a successful path which propped up my career all along.

I wish to express my sincere thanks to all the non-teaching staff members and labourers of **CPBMB and DIC** for their whole-hearted cooperation and timely assistance. I also appreciate and acknowledge the facilities provided at **CPBMB** and **Bioinformatics Centre** for the successful completion of my research work. I express my sincere gratitude to all the non-teaching staff members and laborers of **Amala Cancer Research Center** for their timely assistance and facilities provided.

I express my deep sense of gratitude to **Kerala Agricultural University** for financial and technical support for persuasion of my study and research work.

I owe special thanks to **Central Library (Kerala Agricultural University)** and **Library (College of Horticulture). Dr. Francis** and all other staff members of Library, who guided me in several ways, which immensely helped for collection of literature for writing my thesis.

I am forever indebted to my family **Achan, Amma, husband and in-laws** for their constant prayers, unfathomable love and boundless affection. I thank **my husband** for his unwavering love, patience and support which strengthen me to stay at tough tracks. Words can't express my soulful gratitude to my **Achan and Amma** for their selfless sacrifice, unconditional love and constant prayers. I am affectionately dedicating this thesis to them.

Devi Lekshmi S

Dedicated to my beloved
PARENTS and GUIDE

TABLE OF CONTENTS

CHAPTER	TITLE	PAGE NO.
1	INTRODUCTION	1
2	REVIEW OF LITERATURE	4
3	MATERIALS AND METHODS	25
4	RESULTS	53
5	DISCUSSION	109
6	SUMMARY	136
7	REFERENCES	I-XVIII
8	ANNEXURES	I-II
9	ABSTRACT	I-III

LIST OF TABLES

TABLE NO.	TITLE	PAGE NO.
1	Fatty acid composition in coconut oil	10
2	List of chemicals and plastic wares used for wet lab studies	28
3	Drug likeliness properties of ligands as per Veber and Lipinski's rule	54
4	Details of different protein targets identified for the diseases	55
5	Details of active site and PDB ID of protein targets involved in carcinoma	61
6	Details of FDA approved drugs in use for cancer	62
7	Dock scores of coconut compounds and the commercial drugs against the protein target EGFR identified for cancer	64
8	Dock scores of coconut compounds and the commercial drugs against protein target CDK identified for cancer	65
9	Dock scores of coconut compounds and commercial drugs against the protein target DHFR identified for carcinoma	67
10	Dock scores of coconut compounds and commercial drugs against the target TS identified for carcinoma	70
11	Dock scores of coconut compounds and commercial drugs against the target VEGFR identified for carcinoma	71
12	Dock scores of coconut compounds and commercial drugs against the protein target ER identified for carcinoma	73
13	Dock scores of coconut compounds and commercial drugs against the target Bcl-xl identified for carcinoma	75
14	Details of active site and PDB ID of protein targets involved in alzhemier's	77
15	Details of the FDA approved drugs in use for alzheimer's	77

LIST OF TABLES CONTINUED…

TABLE NO.	TITLE	PAGE NO.
16	Dock scores of coconut compounds and commercial drugs against the target APP identified for alzheimer's	78
17	Dock scores of coconut compounds and commercial drugs against the protein target BACE 1 identified for alzheimer's	80
18	Dock scores of coconut compounds and commercial drugs against the protein target BACE 2 identified for alzheimer's	81
19	Details of the active site and PDB ID of protein targets involved in diabetes	84
20	Details of the FDA approved drugs in use for diabetes	84
21	Dock scores of coconut compounds and commercial drugs against the protein target ALDR identified for diabetes	86
22	Dock scores of coconut compounds and commercial drugs against the protein target DPP-IV identified for diabetes	89
23	Details of the active site and PDB ID of protein targets involved in hyperlipidemics	91
24	Details of the FDA approved drugs in use for hyperlipidemics	91
25	Dock scores of coconut compounds and commercial drugs against the protein target HMGCo A reductase identified for hyperlipidemics	93
26	Dock scores of coconut compounds and commercial drugs against the target CEase identified for hyperlipidemics	96
27	ADME- Toxicity scores of different coconut phyto compounds	98
28	ADME- Toxicity scores of commercial drugs used for the treatment of selected diseases	100

LIST OF TABLES CONTINUED...

TABLE NO.	TITLE	PAGE NO.
29	Serum lipid profile (mg/dl) of male Sprague dawley rats fed with coconut oil, lauric acid and atorvastatin, the standard drug	102
30	Liver enzymatic profile (U/ mg Protein) of male Sprague dawley rats fed with coconut oil, lauric acid and atorvastatin, the standard drug	105
31	Ct and ΔΔCt values obtained for the different genes through qPCR	108

LIST OF PLATES

PLATE NO.	TITLE	BETWEEN PAGES
1	3D structures of selected proteins identified for carcinoma before and after preparation using Discovery studio	59
2	Hydrogen bond interactions between capric acid and CDK	65
3	Hydrogen bond interaction between lauric acid and DHFR	69
4	Hydrogen bond interaction between lauric acid and TS	69
5	Hydrogen bond interaction between lauric acid and BACE 1	81
6	Hydrogen bond interaction between lauric acid and BACE 2	82
7	Hydrogen bond interaction between lauric acid and Aldose reductase	87
8	Hydrogen bond interaction between lauric acid and HMG Co reductase	94

LIST OF FIGURES

FIGURE NO.	TITLE	BETWEEN PAGES
1	Composition of different MCFAs in coconut oil	10
2	Cytotoxicity induced by lauric acid on HCT 15 cell lines	107
3	Cytotoxicity induced by lauric acid on HepG2 cell lines	107
4	Cytotoxicity induced by lauric acid on RAW 264.7 cell lines	108
5	Interaction of (-ve Binding energy) phyto compounds with the different targets of carcinoma	115
6	Interaction of (-ve Binding energy) commercial drugs with the different targets of carcinoma	115
7	Deviation in CDOCKER and CDOCKER interaction energy observed during interaction of phyto compounds with different targets of carcinoma	116
8	Deviation in CDOCKER and CDOCKER interaction energy observed during interaction of commercial drugs with different targets of carcinoma	116
9	Interaction (-ve Binding energy) of phyto compounds and commercial drugs with different targets of alzheimer's	119
10	Deviation in CDOCKER and CDOCKER interaction energy observed during interaction of phyto compounds and commercial drugs with different targets of alzheimer's	119
11	Interaction (-ve Binding energy) of phyto compounds and commercial drugs with different targets of diabetes	122
12	Deviation in CDOCKER and CDOCKER interaction energy observed during interaction of phyto compounds and commercial drugs with different targets of diabetes	122

LIST OF FIGURES CONTINUED...

FIGURE NO.	TITLE	BETWEEN PAGES
13	Interaction (-ve Binding energy) of phyto compounds and commercial drugs with different targets of hyperlipidemics	124
14	Deviation in CDOCKER and CDOCKER interaction energy observed during interaction of phyto compounds and commercial drugs with different targets of hyperlipidemics	124
15	Serum lipid profile (mg/dl) of male Sprague dawley rats showing the hypolipidemic activity of coconut oil, lauric acid and atorvastatin	129
16	Liver enzymatic profile (U/ mg Protein) of male Sprague dawley rats showing the hypolipidemic activity of coconut oil, lauric acid and atorvastatin	129
17	Liver enzymatic profile (U/ mg Protein) of male Sprague dawley rats showing the anti-diabetic property of coconut oil, lauric acid and atorvastatin	131
18	Amplification plot obtained from *qRTPCR* analysis for the genes Aldose reductase and EGFR against actin as reference gene	134
19	Effect of lauric acid on expression of genes Aldose reductase and EGFR (*qRTPCR*)	134

LIST OF ANNEXURES

SL. NO.	TITLE
1	Coconut compounds used as ligands in the molecular docking analysis and their PubChem ID
2	List of gene specific primers used in *qRTPCR* analysis

ABBREVIATIONS

%	Percentage
>	Greater than
β	Beta
°C	Degree Celsius
μg	Microgram
μl	Microlitre
ADMET	Absorption Distribution Metaboilsm Elimination Toxicity
ALDR	Aldose Reductase
APP	Amyloid Precursor Protein
BACE	Beta Secretase
CDK	Cyclin Dependent Kinase
CEase	Cholesterol Esterase
CHARMM	Chemistry at HARvard Macromolecular Mechanics
cm	Centimetre
CO	Coconut oil
CPBMB	Centre for Plant Biotechnology and Molecular Biology
CYP2D6	Cytochrome P450 2D6
DHFR	Dihydro Folate Reductase
DIC	Distributed Information Centre
DPP	Dipeptidyl Peptidase
EDTA	Ethylene Diamine Tetra Acetic acid
EGFR	Epidermal Growth Factor Receptor
ER	Estrogen Receptor
FBS	Fetal Bovine Serum
g	Gram
ha	Hectare
HDL	High Density Lipoprotein
L	Litre
LDL	Low Density Lipoprotein

LHD	Lauric acid High Dose
LLD	Lauric acid Low Dose
M	Molar
MCFAs	Medium Chain Fatty Acids
mg	Milligram
ml	Millilitre
mM	Milli mole
MTT	3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide
NADH	Nicotinamide Adenine Dinucleotide
NADPH	3-hydroxy-3-methylglutaryl-coenzyme A reductase
TS	Thymidylate Synthase
OD	Optical Density
PBS	Phosphate-buffered saline
PCR	Polymerase Chain Reaction
PDB	Protein Data Bank
qRTPCR	quantitative Real Time PCR
RNA	Ribonucleic acid
RPMI	Rosewell Park Memorial Institute
U	Unit
UV	Ultra violet
V	Volts
VEGFR	Vascular Endothelial Growth Factor Receptor

Introduction

1. INTRODUCTION

Coconut [*Cocos nucifera* L.] often referred as Kalpavriksha, is one of the most important oil-yielding crops of the world grown in more than 80 countries of the tropics. Coconut palm exerts a profound influence on the rural economy of many states where it is grown extensively and it provides sustenance to more than 10 million people in India. Coconut production in Kerala plays an important role in the state economy and culture. It has been an important component of the diet of Kerala population for decades. It is widely acknowledged as a multipurpose tree (Enig, 2004).

Coconut provides nutritious source of kernel, juice, milk, and oil that has fed and nourished populations around the world for generations. Coconut oil is the healthiest oil on earth which provides the primary source of fat in the diets. Coconut oil is of special interest because it possesses healing properties far beyond that of any other dietary oil and is extensively used in traditional medicine among Asian and Pacific populations. Ninety two per cent of coconut oil comprises of saturated fatty acids in which 70 per cent are Medium Chain Fatty Acids (MCFAs). Medium chain fatty acids in coconut oil have been recognized for its unique properties related to its antiviral, antibacterial and antiprotozoal functions. MCFAs in coconut include lauric, myristic, capric and caprylic acids. Among these, 50 per cent of MCFAs is contributed by lauric acid. Almost all the MCFAs used in research, medicine and food products come from coconut oil.

Being rich in saturated fatty acids (SFA) coconut oil is bracketed with animal fats having cholesterogenic properties, creating a fear complex about consumption of coconut oil resulting in elevated cholesterol, diabetic complications and other health issues. However even after years of study, researchers are unable to link coconut oil consumption with an increased risk of heart problems. In fact, evidences show that coconut oil may actually protect against heart diseases (Papamandjaris *et al.*, 1998).

The coconut oil consumption for cooking purpose even dropped to 25000 tonnes by the end of 80s because of the malicious propaganda unleashed by the refined vegetable oil manufacturers that the consumption of coconut oil will increase cholesterol level in the body. Controversy about the coconut oil consumption, weather it is beneficial or hazardous to health exists for a long time. Scientific community is still trying to clear these controversies but research reports for scientific background are not yet available. People are still confused in the consumption of this natural oil.

In 1980 Hostmark *et al.,* reported that coconut oil feeding produced significantly higher alpha lipoproteins (HDL), the good cholesterol relative to sunflower oil feeding. Similarly Award (1981) claimed that total tissue cholesterol accumulation for animals on the safflower diet was six times greater than on coconut oil diet and twice in that of soybean oil diet.

The incidence of Coronary Heart Disease (CHD) could be better linked with consumption of cholesterol rich animal originated food and the sedentary habits associated with a tension ridden mechanical society. Moreover it may not be the type of cooking oil, rather its quantity that may be contributing to the risk of coronary artery disease (Tsuji, 2001). .

The present study, "Molecular docking and validation of medicinal effects of coconut (*Cocos nucifera* L.)" aims at enlightening the medicinal effects of coconut through different approaches like *in silico* and *in vivo* analysis. Molecular docking is a technique which predicts the preferred orientation of one molecule to a second when bound to each other to form a stable complex (Pratheepa, 2012). This docking technique can be performed with different tools like Discovery studio, Auto dock etc. Computational techniques can strongly support designing novel, more potent inhibitors by revealing the mechanism of drug-receptor interaction (Tiwari *et al.*, 2012). Molecular docking approaches are routinely used in modern drug designing to

identify small molecules by orienting and scoring them in the active binding site of a protein.

In silico computational tools can be used to screen out the medicinal properties of coconut phyto compounds mainly MCFAs and its potential to get converted into drugs for health benefits. *In silico* results obtained could be further validated using wet lab methods. Thus, effort was made in the present study, to analyze the medicinal effects of important components in coconut through *in silico* analysis and its validation through wet lab studies. Effect of various components to inhibit or activate the targets involved in cardiovascular disorders, cancer, alzheimer's, diabetes etc. were analyzed through molecular docking and the positive results were validated in animal models, cell line cultures and expression analysis of the gene concerned.

REVIEW OF LITERATURE

2. REVIEW OF LITERATURE

2.1 GENERAL BACKGROUND

Coconut is cultivated for its multiple utilities and is mainly valued for its nutritional and medicinal properties. It was known as Nuxindica in the medieval period. According to Woodroof (1970), early Spanish explorers called it coco, which means "monkey face" because the three indentations (eyes) on the hairy nut that resemble the head and face of a monkey. Botanically coconut is a drupe and not a nut. The coconut palm is a monocotyledon and belongs to the order Arecaceae, family Palmae and the specie is known as *Cocos nucifera L.* Nucifera means "nut-bearing". The coconut plant is monoecious, producing both male and female flowers. The male flowers are located distally while the female flowers are proximal on each inflorescence.

Coconut is believed to have its origin in the Indo-Malayan region, from where it spread throughout the tropics (Kumar, 2008). Its natural habitat was the narrow sandy coast, but is now found on soils ranging from pure sand to clays and from moderately acidic to alkaline. Area, production and productivity status of coconut in India in the year of 2013-14 are 2140.50 ha, 21665.19 million tons and 10122 nuts per ha respectively. In India coconut is mostly cultivated in the coastal regions of the country. The states that have abundant coconut growth are Andhra Pradesh, Assam, Goa, Karnataka, Kerala, Maharashtra, Orissa, Tamil Nadu, Tripura, West Bengal, Andaman and Nicobar Islands, Lakshadweep and Pondicherry. Among these Kerala accounts for the largest area (797.21 ha) and production (5968.01 million tons) of coconut in India (CDB, 2014).

Coconut trees generally start to produce fruit five to six years after planting (Thampan, 1981). Mature trees produce their fruits year-round rather than in one specific season. According to Pacific Island Agroforestry, a well-maintained coconut palm will produce 80 to 100 nuts in a year.

Coconut varieties fall under two broad groups, Tall or typica and Dwarf or nana. Tall and Dwarf coconut types may hybridize to produce intermediate forms (Woodroof 1970).The tall varieties normally live for over 60 years and are adaptable to a wide range of soil conditions. They start bearing within six to ten years. The Dwarf varieties start bearing within three to four years attain full production by the ninth year and have an economic life span of about 30 to 40 years (Woodroof, 1970).

2.1.1 Social and economic importance of coconut

The social and economic importance of coconut could be traced back since the 19th century by the arrival of Europeans in the Pacific. Coconut oil was the first vegetable oil to appear in world trade and it caused wide and fast commercialization of the plant (Chan and Elevitch, 2006).

In India, the use of coconut for food, and its applications in the Ayurvedic medicine are documented in Sanskrit literature about 4000 years ago. Coconut palm is known as *Kalpavriksha* in Sanskrit, meaning "tree which gives all that is necessary for living," since nearly all parts of the tree can be used in some manner or other (Ahuja and Ahuja, 2014).

Each part of coconut tree is beneficial for human and is also a source of remarkably diverse products (Fife, 2003). Fruit is a rich source of juice, edible kernel, oil, shell and fiber whereas frond and trunk can be used as the building materials. Heart-of-palm at the growing point is edible. Coconut sap (toddy) is beneficial for drinking and is a good source of sugar. All of these provide the rationale for the title "The Tree of Life" respectfully bestowed upon this palm (Harries and Paull, 2008). Industrial uses further include the separation of glycerin and fatty acids, the production of soaps, detergents and bio-diesel, and Coco Methyl Ester (CME) for mixing with regular diesel.

A whole coconut consists of 50 per cent husk, 15 per cent shell, 25 per cent meat and 10 per cent water. Coconuts are different from any other fruits

and nuts as they contain a large quantity of "water" and when immature they can be used fresh or preserved as soft drink (Mandal and Mandal, 2011). Tender coconut water is one of the world's most popular and valuable natural drinks. Coconut kernel and its many derivatives have immense benefit in the human diet, giving energy and sustaining good health. The husk provides a durable fiber for mat and rope making. The shell is especially energy-dense and hard, making it a valuable fuel and a durable material for tools, ornaments and curios.

Coconut kernel in its raw and processed form has long been used as a vital, delicious ingredient in cakes and confectioneries. It is a compatible partner to chilli spiced dishes in traditional diets of India, Thailand and the Philippines. Coconut oil has a multitude of uses: as cooking oil, in body and hair lotions, in medication for abrasions, skin rashes and burns; as lighting and engine fuel oil; and as a feedstock for soaps and detergents (Hegde, 2006).

2.2 COCONUT OIL

According to Mandal and Mandal (2011), coconut oil is the healthiest oil on earth which has been consumed in tropical countries for thousands of years. In Kerala production of coconut oil seems to be the basic intention behind coconut cultivation.

It has a long shelf life and a melting point of 24 °C. Coconut oil is produced by crushing and pressing copra, the dried kernel, which contains about 60 to 65 per cent oil. Mainly two methods have been developed to extract coconut oil, either through dry or wet processing. Dry processing is the most widely used form of extraction of coconut oil (Grimwood *et al.*, 1975). Clean, ground and steamed copra is pressed by wedge press, screw press or hydraulic press to obtain coconut oil, which is then directly used or further refined through bleaching and deodorizing (RBD) processes.

Wet process uses raw coconut rather than dried copra. The protein in the coconut creates an emulsion of oil and water (Hamid *et al.*, 2011). Wet processing

is less viable than dry processing due to a 10 to15 per cent lower yield, even compared to the losses due to the spoilage and pests with dry processing. Wet processes also require investment on equipment and energy, incurring high capital and operating costs (Grimwood *et al.*, 1975).

Coconut oil has the natural sweet taste and contains 92 per cent of saturated fatty acids mainly Medium Chain Fatty Acids (Fife, 2005). All fats and oils are composed of fat molecules called fatty acids. There are two methods of classifying fatty acids. The most familiar is based on saturation. They are saturated fatty acids, monounsaturated fatty acids, and polyunsaturated fatty acids. The second method of classification is based on molecular size or length of the carbon chain in the fatty acid, short-chain fatty acids (SCFA), medium-chain fatty acids (MCFA) and long-chain fatty acids (LCFA).

The vast majority of the fats and oils that we eat, whether they are saturated or unsaturated or of animal/ plant origin are composed of long-chain fatty acids. Probably 98 to 100 per cent of all the fats we eat consist of long chain fatty acids. Coconut oil is unique since it is composed predominately of Medium Chain Fatty Acids (Gervajio, 2005).

Malongil *et al.* (2009) claimed that the size of the fatty acid is extremely important to determine its function. Medium-chain fatty acids are absorbed and transported directly to the liver where they are burned for energy and are not getting stored. Long-chain fatty acids are converted into triglycerides and then are taken up by cells and used for energy or stored as the fatty deposits. Long chain fatty acids become part of protein particles and travel in the bloodstream to be taken up by cells and the liver. Bile from the gallbladder is needed to digest long chain fatty acids. The physiological effects of medium-chain fatty acids in coconut oil are distinctly different from that of the long-chain fatty acids. This makes MCFAs in coconut oil differ from all other fats and give it its unique character and healing properties.

Coconut oil is antiviral, antifungal and antibacterial in action. It attacks and kills viruses causing herpes, HIV, hepatitis C, the flu, and mononucleosis having lipid fatty coating. Coconut oil can be used against pneumonia, sore throats, dental cavities, urinary tract infections, meningitis, gonorrhea, food poisoning and many more bacterial infections (Cohen, 1986). Also it is a good remedy for fungus/yeast infections that cause candida, ringworm, athlete's foot, thrush, jock itch and diaper rash.

2.2.1 Propaganda against coconut oil

The demand for coconut oil for cooking purposes started declining with the availability of palm oil at low price and with the entry of so called 'heart-friendly' refined vegetable oils in attractive packets at reasonable rates in local markets. The coconut oil consumption for cooking purpose even dropped to 25000 tonnes by the end of 80s because of the malicious propaganda unleashed by the refined vegetable oil manufacturers that the consumption of coconut oil will increase cholesterol level in the body (Nandakumar, 1998). Though there were no scientific data to relate consumption of coconut oil on increased cholesterol level, the propaganda had serious impact on the consumers.

Coconut is one of those foods that seems to ping-pong between the 'good food' and 'bad food' list. Both the American Heart Association and the National Heart Foundation recommended avoiding the use of coconut oil for cooking, but both their websites include recipes that contain coconut milk, albeit a reduced-fat version (Nogrady, 2008). Despite the fuzzy perception that all things from plant must be better for human, oil made from coconuts actually contains 85 to 92 per cent saturated fat. Saturated fats, usually the dominant type in animal foods, are generally regarded as the baddies when it comes to heart disease. Even now people get confused to use the coconut oil whether it is good for their body or not. Scientists were failed to provide detailed scientific data to overcome this controversies and they are still working on this subject.

2.2.2 Composition of coconut oil

The coconut oil contains 92 per cent of saturated fatty acids in which 72 per cent are short chain saturated fatty acids known as medium chain fatty acids (MCFAs). In contrast to animal fat coconut oil has no cholesterol. Composition of fatty acids present in coconut oil are given in Table 1. The dried kernel of coconut contains 60 to 65 per cent oil. Once the oil is extracted, the remaining coconut cake is 18 to 25 per cent protein but contains so much dietary fiber and cannot be eaten in large quantities by humans. Instead it is normally fed to ruminants (Grimwood *et al.*, 1975).

There are two types of saturated fatty acids present in coconut. They are, Medium Chain Fatty Acids with carbon length of 3 to 16 and Long Chain Fatty Acids with carbon length of more than 16 (Thampan, 1994). Medium Chain Fatty Acids (MCFAs) are supposed to assimilate well in the body's system. Lauric acid (52%) is the chief contributor, followed by capric acid (10%), caprylic acid (9%) and myristic acid (19%) (Figure 1). Stearic acid (11%) and palmitic acid (8%) are some of the long chain fatty acids present in coconut.

Other than MCFAs, coconut oil is composed of poly-phenols like gallic acid and caffeic acid which are also known as phenolic acids. These polyphenols are responsible for the fragrance and the taste of coconut oil. Virgin coconut oil is reported to be rich in these polyphenols (Sharma, 2014).

2.3 MEDIUM CHAIN FATTY ACIDS IN COCONUT OIL

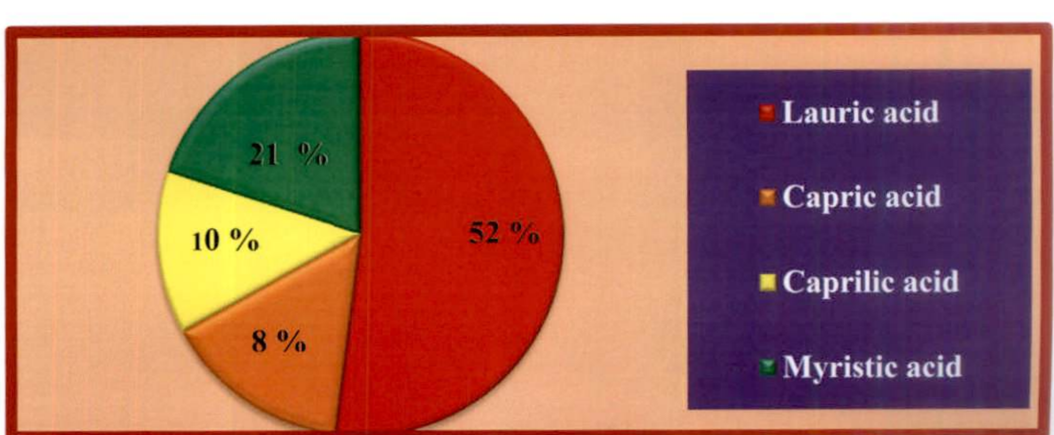
2.3.1 Lauric acid

Lauric acid or systematically dodecanoic acid is a medium chain saturated fatty acid with a 12-carbon chain. It is a white powdery solid with a molecular formula $C_{12}H_{24}O_2$. Lauric acid comprises about half (52 %) of the fatty acid content in coconut oil. It is also found in human breast milk (6.2 %), cow's milk (2.9 %), and goat's milk (3.1 %) (Anneken *et al.*, 2006).

Table 1: Fatty acid composition in coconut oil (Gervajio, 2005)

Sl no.	Name of fatty acid	No. of carbon present	Percentage (%)	Remarks	Type of fat
1	Lauric acid	C12:0	45 to 52	Medium chain fatty acid	Saturated fat
2	Myristic acid	C14:0	16 to 21	Medium chain fatty acid	Saturated fat
3	Caprylic acid	C8:0	5 to 10	Medium chain fatty acid	Saturated fat
4	Capric acid	C10:0	4 to 8	Medium chain fatty acid	Saturated fat
5	Palmitic acid	C16:0	7 to 10	Long chain fatty acid	Saturated fat
6	Oleic acid	C18:0	5 to 8	Monounsaturated fatty acid	Unsaturated fat
7	Linoleic acid	C18:0	1 to 3	Poly unsaturated fatty acids	Unsaturated fat
8	Linolenic acid	C18:0	Up to 0.2	Poly unsaturated fatty acids	Unsaturated fat
9	Stearic acid	C18:0	2 to 4	Long chain fatty acid	Saturated fat

Figure 1: Composition of different MCFAs in coconut oil



Thijssenn *et al.* (2005) reported that the human body converts lauric acid into monolaurin, which is supposedly helpful in dealing with viruses and bacteria that cause diseases such as herpes, influenza, cytomegalovirus, and even HIV. It also helps in fighting harmful bacteria such as *Listeria monocytogenes* and *Helicobacter pylori* and harmful protozoa such as *Giardia lamblia. Giardia lamblia* is a common cause of diarrheal disease in humans, particularly among children causing nutritional disorders. Aly *et al.* (2013) reported that lauric acid combined with metronidazole had positive therapeutic effect against giardiasis.

Kabara (1972) have also recognized the antimicrobial activity of the monoglyceride of lauric acid called monolaurin.

Mensink *et al.* (2003) characterized lauric acid as having "a more favorable effect on total HDL cholesterol than any other fatty acid, either saturated or unsaturated". It increases total serum cholesterol and decreases the ratio of total to HDL cholesterol. But most of the increase is attributable to an increase in high-density lipoprotein (HDL), the "good" blood cholesterol.

2.3.2 Caprylic acid

Caprylic acid is the common name for the eight-carbon saturated medium chain fatty acid, octanoic acid. Its compounds are found naturally in the milk of various mammals, and as a minor constituent of coconut oil and palm kernel oil. Its molecular formula is $C_8H_{16}O_2$.

Caprylic acid is used by holistic practitioners to treat yeast infections, as well as to restore healthy pH levels in the body. Hoshimoto *et al.* (2002) reported that caprylic acid shows promises in the treatment of Crohn's disease. They help in treating this disease by suppressing the release of interleukin 8, a protein that plays a key role in promoting intestinal inflammation. Caprylic acid may also help to keep the cholesterol level low. In vitro tests conducted on rats determined that treatment with caprylic acid led to a decrease in cholesterol levels, but it failed to lower the blood pressure (Kim *et al.*, 2008).

2.3.3 Capric acid

As per IUPAC, capric acid is referred as decanoic acid and its molecular formula is $C_{10}H_{20}O_2$. Capric acid is a medium chain fatty acid present in coconut oil and has antimicrobial, antiviral and antifungal properties (Kasai *et al.*, 2003). It is also present in breast milk and protects the baby from bacterial, viral and fungal infections.

In the body capric acid, reacts with certain enzymes secreted by bacteria, which subsequently convert into a powerful antimicrobial agent called monocaprin. Angadi (2012) reported that monocarpin and doxycycline, a tetracycline antibiotic combination gel may be effective for the treatment of viral disease like *Herpes labialis*.

Ogbolu *et al.* (2007) reported that coconut oil can be used for the treatment of Candida where the capric acid has been found very effective in killing the yeast.

2.3.4 Myristic acid

Myristic acid, also called tetradecanoic acid, is a saturated medium chain fatty acid with the molecular formula CH_3 $(CH_2)_{12}COOH$. A myristate is a salt or ester of myristic acid. Myristic acid is named after the nutmeg *Myristica fragrans*.

2.4 MEDICINAL EFFECTS OF MEDIUM CHAIN FATTY ACIDS IN COCONUT OIL

Coconut is kind of an "exotic" food in the Western world, primarily consumed by health conscious people. It is a dietary staple that people have thrived on for many generations. Prior *et al.* (1981) had reported about the Tokelauans, a population which live in the South Pacific, eat over 60 per cent of their calories from coconuts and are the biggest consumers of saturated fat in the world. These people are in excellent health, with no evidence of heart disease.

Another example of a population that eats a lot of coconut and remains in excellent health is the Kitavans, the Trobriand Islands group of Papua New Guinea (Lindeberg and Lundh, 1993).

Stubbs and Harbron (1996) conducted a study in which varying amounts of medium and long chain fatty acids were fed to human and found out that the uptake of food by those who ate maximum MCFAs were much lower than that of others. Wymelbeke *et al.* (1998) reported that those who ate the most MCFAs at breakfast ate significantly fewer calories at lunch. The unique property of coconut oil to reduce hunger may be related to the way the medium chain fatty acids in it are metabolized, since ketone bodies can have an appetite reducing effect (Mcclernon *et al.*, 2007).

These studies were small and only done for a short period of time. If this effect were to persist over the long term, it could have a dramatic influence on body weight over a period of several years.

Obesity is currently one of the biggest health problems in the world. Obesity is not only a matter of calories, but also the sources of those calories are critical too. Different foods affect our bodies and hormones in different ways. The medium-chain MCFAs in coconut oil can increase energy expenditure compared to the same amount of calories from longer chain fats (Seaton *et al.*, 1986 and Scalfi *et al.*, 1991). Dulloo *et al.* (1996) found that 15-30 grams of MCFAs per day increased 24 hour energy expenditure by 5 per cent, totaling about 120 calories per day.

Coconut oil has been demonized in the past because it is the one of the richest sources of saturated fat (90 %) known to man. But new research data is showing that saturated fats are harmless. Many massive studies that include hundreds of thousands of people prove that the whole "artery-clogging" idea with saturated fat was a myth (Siri-Tarino *et al.*, 2010).

Coconut oil is loaded with saturated fats, which actually do not harm the blood lipid level. Mensink *et al.* (2003) claimed that saturated fats raise HDL (the good) cholesterol and change the LDL cholesterol to a benign subtype. Nevin and Rajamohan (2004) conducted rat studies showing that coconut oil reduces triglycerides, total and LDL cholesterol, increases HDL and improves blood coagulation factors and antioxidant status. This improvement in cardiovascular risk factors should theoretically lead to a reduced risk of heart disease over the long term. Coconut oil reduced total and LDL cholesterol in women while increased HDL level compared to soybean oil (Assuncao *et al.*, 2009).

Coconut oil does not contain average run-of-the-mill saturated fats found in cheese or steak. Instead, it contains so called MCFAs which are fatty acids of a medium length. Most of the fatty acids in the diet are long-chain fatty acids, but the medium-chain fatty acids in coconut oil are metabolized differently. They go straight to the liver from the digestive tract, where they are used as quick source energy or turned into ketone bodies, which can have therapeutic effects on brain disorders like epilepsy and alzheimer's (Veech *et al.*, 2001).

The best known therapeutic application of ketogenic diet is treating drug-resistant epilepsy in children (Neal *et al.*, 2008). Ketogenic diet involves eating very little carbohydrates and large amounts of fat, leading to greatly increased concentrations of ketone bodies in the blood. This diet can dramatically reduce the rate of seizures in epileptic children, even those who haven't had success with multiple different types of drugs. Because the MCFAs in coconut oil get shipped to the liver and turned into ketone bodies, they are often used in epileptic patients to induce ketosis while allowing for a bit more carbohydrates in the diet (Liu, 2008).

MCFAs in coconut oil is good in curing neurological disorders characterized by the death of neurons including alzheimer's diseases, parkinson's diseases and may also be protective in traumatic brain injury and stroke (Kim *et al.*, 2005). Alzheimer's disease is the most common cause of dementia worldwide

and occurs primarily in elderly individuals. In alzheimer's patients, there appears to be a reduced ability to use glucose for energy in certain parts of the brain. Ketone bodies can supply energy for the brain and researchers have speculated that ketones can provide an alternative energy source for these malfunctioning cells and reduce symptoms of Alzheimer's (Costantini *et al.*, 2008).

Alzheimer's patients consumed a beverage containing MCFAs or a beverage without MCFAs were undergone a cognitive test after 90 minutes and found out that those patients who received the MCFAs scored significantly better scores on the test than the other group (Reger *et al.*, 2004). Other studies support these findings and MCFAs are being intensively studied as potential therapeutic agents in Alzheimer's disease (Henderson *et al.*, 2009).

A ketogenic diet amounts to nothing less than a drug-free cancer treatment (Nebeling *et al.*, 1995). MCFAs, LCFAs, and their mixture were compared in reference to both cytotoxic effect against human tumor cells and influence on the immune system. MCFAs showed more potent cytotoxicity than LCFAs. Continuous contact with MCFAs also inhibited the cytotoxic effect of lymphokine-activated killer (LAK) cells much more strongly than LCFAs (Kimoto *et al.*, 1998). The diet calls for eliminating carbohydrates, replacing them with healthy fats and protein. The MCFA lauric acid was effective at inducing apoptosis in the Caco-2 cell line via a p53 independent pathway (Fauser *et al.*, 2013).

Coconut oil can serve various purposes that have nothing to do with eating it. Many people are using it for cosmetic purposes and to improve the health and appearance of their skin and hair. Coconut oil when applied on individuals with dry skin is reported to improve the moisture and lipid content of the skin (Agero and Rowell, 2004).

Coconut oil can also be very protective against hair damage. It is effective as sunscreen, blocking about 20 per cent of the sun's ultraviolet rays (Rele and Mohile, 2003). Another application is using it like mouthwash in a process

called oil pulling, which can kill some of the harmful bacteria in the mouth, improve dental health and reduce bad breath (Asokan *et al.*, 2011).

2.5 POLY PHENOLS IN COCONUT OIL

In addition to the saturated fatty acids, coconut oil contain poly phenols which include gallic acid, caffeic acid etc. Total phenolic contents of the virgin coconut oil reported is 48.17–57.89 mg GAE/100 g oil (GAE- Gallic Acid Equalent). Vysakh *et al.* (2014) demonstrated the potential beneficiary effect of polyphenols extracted from virgin coconut oil on adjuvant induced arthritis in rats through antioxidant and anti-inflammatory effects.

Gallic acid is a trihydroxy benzoic acid, a type of phenolic acid, also known as 3,4,5-trihydroxybenzoic acid. Per gram of coconut oil contain 6.29 to 8.38 mg gallic acid. Gallic acid seems to have anti-fungal and anti-viral properties. Gallic acid acts as an antioxidant and helps to protect our cells against oxidative damage. Gallic acid was found to show cytotoxicity against cancer cells, without harming healthy cells (Karimi, 2013). Gallic is also used to treat albuminuria and diabetes. Some ointment to treat psoriasis and external hemorrhoids contains gallic acid.

2.6 VIRGIN COCONUT OIL

Virgin coconut oil is extracted from coconut milk by a "wet" process under controlled temperatures, which helps to protect beneficial components such as polyphenols and tocopherols. The term virgin refers only to the process and not on the chemical properties which are essentially the same in both RBD oil and VCO. It is the purest form of coconut oil, water white in colour. Their effect on health would likewise be the same, given the same medium chain fatty acid (MCFA) compositions. Virgin coconut oil (VCO) is abundant in vitamins, minerals and anti-oxidants, thus making it the 'mother of all oils' (Mansor *et al.*, 2012). The amount of gallic acid is high in VCO compared to RBD coconut oil.

Based on preliminary animal research, some have suggested that the phytochemicals in virgin coconut oil may support health through antioxidant and anti-inflammatory action (Nevin and Rajamohan, 2010). Rich in Vitamin C and Vitamin E, virgin coconut oil slows down the ageing process and assures the best of life and beauty to your skin (Carandang, 2008). It also helps in the absorption of fat soluble vitamins A, D, E and K. Virgin coconut oil is completely nontoxic to humans, and is referred to as the "drug store in a bottle".

2.7 PLANT DERIVED DRUGS

Nature, the master craftsman of molecules created almost an inexhaustible array of molecular entities. Mishra *et al.* (2013) suggested that having different biosynthetic pathways plants are great sources of natural compounds, which can be used for various therapeutic purposes.

The World Health Organization estimates that 80 per cent of people in the developing countries rely on traditional medicine for their primary health care, and about 85 per cent of traditional medicine involves the use of plant extracts. This means about 3.5 to 4 billion people in the world rely on plants as sources of drugs (Farnsworth and Soejarto, 1985). Phytochemicals are a large group of plant-derived compounds and are considered as a potential alternative cure in medical treatment (Arts and Hollman, 2005). Veeresham (2012) claimed that natural products have been the backbone of traditional system of healing throughout the globe, and have also been an integral part of history and culture. The use of bioactive natural products as herbal drug preparations dates back hundreds and thousands of years ago but their application as isolated and characterized compounds to modern drug discovery and development started only in the 19th century.

Examples of important drugs obtained from plants are digoxin from Digitalis spp., quinine and quinidine from Cinchona spp., vincristrine and vinblastine from *Catharanthus roseus*, atropine from *Atropa belladonna* and morphine and codeine from *Papaver somniferum*. Betulinic acid from *Betula*

alba, camptothecin from *Camptotheca acuminata,* podophyllotoxin from *Podophyllum peltatum* and topotecan from *Camptotheca acuminata* are some of the commonly used drugs for cancer treatment which are of plant origin (Taylor, 2000). It is estimated that 60 per cent of anti-tumour and anti-infectious drugs already on the market or under clinical trial are of natural origin (Harvey, 2008).

According to Lutz (2003), natural products not only complement synthetic molecules, but also exhibit drug- relevant features unsurpassable by any synthetic compound. The main aim of a medicinal chemist is to get active extracts, fractions or compounds against a particular target. Thus the natural products which have found direct medicinal application as drug entities, can also serve as chemical models or templates for the design, synthesis, and semi synthesis of novel substances for treating humankind's diseases (Veeresham, 2012).

In the recent times, computational chemistry and *in silico* analysis have become an economic solution for drug discovery and to identify the lead molecules (Mukhtar *et al.*, 2008). Similar *in silico* techniques can be used to analyze the medicinal properties claimed by the coconut and its constituents and can check the potential of these compounds to get transformed into drugs.

2.8 SIGNIFICANCE OF BIOINFORMATICS

Biological data are being produced at a phenomenal rate (Reichhard, 1999). Genome projects are scientific endeavors that ultimately aim to determine the complete genome sequence of an organism and to annotate protein-coding genes and other important genome-encoded features. Till now scientists were able to identify genome information of 14111 organisms which include 172 plants and 600 animals. To the field of medicine, these informations will help to discover potential cures to various diseases. In agriculture, these studies pave the way to understand plant evolution, and use this knowledge to improve crops.

As of 19 March 2014, release "2014_03" of UniProtKB/Swiss-Prot contains 542,782 protein sequence entries and release "2016_01" of

UniProtKB/TrEMBL contains protein 60,268,458 sequence entries (Expasy, 2016). Similarly, as of 15 August 2015, GenBank release 209.0 has 187,066,846 reported nucleotide sequences (NCBI, 2015). These databases are doubling in size every 15 months.

To be able to handle all this genetic information, share and make sense of it, scientists need databases to store the information, where it can be accessed and mined. They also need tools, such as computer software, to manage the information; and algorithms (mathematical formulae) to analyze the information and use it to answer specific questions, such as the location of genes, the structure of proteins, and species relatedness (Sharma and Sarkar, 2012). To do all this (and more), bioinformatic tools are highly essential.

Bioinformatics is a new science that combines the power of computers, mathematical algorithms, and statistics with concepts in the life sciences to solve biological problems. Bioinformatics is seen as an emerging field with the potential to significantly improve how drugs are found, brought to the clinical trials and eventually released to the market place. Computer – Aided Drug Design (CADD) is a specialized discipline that uses computational methods to simulate drug – receptor interactions. CADD methods are heavily dependent on bioinformatics tools, applications and data bases. *In silico* methods can help in identifying drug targets via bioinformatics tools (Pugazhendhi and Umamaheswari, 2013). They can also be used to analyze the target structures for possible binding/ active sites, generate candidate molecules, check for their drug likeness, dock these molecules with the target, rank them according to their binding affinities, further optimize the molecules to improve binding characteristics.

2.9 *IN SILICO* ANALYSIS AND MOLECULAR DOCKING STUDIES

Natural products including phytochemicals have been widely used in the development of drugs. *In silico* research in medicine is thought to have the potential to speed the rate of discovery while reducing the need for expensive lab

work and clinical trials. One way to achieve this is by producing and screening drug candidates more effectively.

In silico is an expression used to mean "performed on computer or via computer simulation". This approach differs from use of expensive high-throughput screening (HTS) in robotic labs to physically test thousands of diverse compounds a day often with an expected hit rate on the order of 1 per cent or less with still fewer expected to be real leads following further testing (Rohrig *et al.*, 2010).

Docking is a computational approach which predicts the preferred orientation of one molecule to a second when bound to each other to form a stable complex (Kitchen, 2004). Docking is frequently used to analyze the binding orientation of drug candidates to their protein targets. A drug is a small molecule that can interact, bind and control the function of biological receptors that helps to cure a disease. Receptors are proteins that interact with other biological molecules to maintain various cellular functions in body. Enzymes, hormone receptors, cell signaling receptors, neurotransmitter receptors etc. are some important receptors in our body.

A binding interaction between the ligand and an enzyme protein may result in activation or inhibition of the enzyme. To cure or control a disease, the expression of the protein resulting in the disease should be suppressed. So the ligand should act as an inhibitor in most cases. The molecules binding to a receptor inhibit its function (Mathew and Raj, 2009) and can be called as a drug.

Many *in silico* studies regarding the medicinal properties of plant derived compounds were already reported. Kumar *et al.* (2012) confirmed the inhibitory nature of plant derived catechin against Porcine Pancreatic Phospholipase A2 through *in silico* analysis. The anti-diabetic activity of Indian medicinal plant, *Gymnema sylvestre,* was analysed through *in silico* studies and reported that the plant component gymnemic acid was effective in reducing diabetic complications (Sahu and Shukla, 2014). Similarly, Mathi *et al.* (2014) confirmed the anti-

cancerous activity of a medicinal plant, *Sophora interrupta* through *in silico* analysis. Barlow *et al.* (2012) evaluated the contents and utilities of traditional Chinese medicines available in the data base using Discovery studio and *in silico* methods.

Similarly, Hariprasd (2011) confirmed the inhibitory nature of gallic acid to PBP2a (the penicillin-binding protein 2a) protein through molecular docking technique using the tool Accelrys Discovery studio. Cleistanthus is a plant genus of the family Phyllanthaceae and is well known for its toxicity. Pratheepa (2012) conducted *in silico* molecular docking analysis using Autodock and found that the compound dioctyl phthalate in the plant exhibited anti-cancerous ability to bind with the p53 receptor.

Shruthila (2014) had done docking studies with the MCFAs of coconut oil against Alzheimer's. The result indicated that these fatty acids bind with Amyloid beta plaque and inhibits its formation. Amyloid beta plaques are the main cause for alzheimer's disease. Mala *et al.* (2015) conducted an *in silico* analysis using the tool Discovery Studio 4 to identify the bioactive compounds to target anti apoptotic proteins- Bcl 2 and Bcl xl. Discovery Studio is a suite of software for simulating small molecule and macromolecule systems. It is developed and distributed by Accelrys, USA.

2.9 WET LAB STUDIES

To predict toxicity, corrosivity, and other safety variables as well as the effectiveness of new products, chemicals, consumer products, medical devices, and also new drugs have involved the use of wet lab methods like *in vivo* animal models and *in vitro* cell culture studies. The compounds that are found to be having potential medicinal values in *in silico* docking analysis are also validated through wet lab experiments to find out their efficiency in a metabolic system.

The idea that all new chemicals and products should be tested for safety in animal studies before being approved for human testing is based on the assumption that animals will respond to drug tests like "little humans". Many *in vivo* research works were already reported highlighting the importance of coconut oil in health benefits. Hostmark *et al.* (1980) found out a significantly lower level of VLDL and significantly higher level of HDL in male wistar rats fed with coconut oil. Rats are considered as a good model organism owing to the larger size of organs and sub organellar structures relative to the mouse (Wallingford *et al.*, 2010). Anti-diabetic and antioxidant effects of virgin coconut oil in alloxan induced diabetic male Sprague Dawley rats were reported (Iranloye *et al.*, 2013). Shariq *et al.* (2015) reported that virgin coconut oil increased the percentage of protection against the anti-atherosclerotic activity in male wistar rats.

Reddy and Maeura (1984) have reported the influence of coconut oil on colon cancer in a group of rats. Cohen *et al.* (1984) induced mammary cancer in test animals which were fed different types of oils. In both these experiments, those fed with coconut oil gave a positive result in curing the disease. Blackburn *et al.* (1989) reported that coconut oil is a neutral fat in terms of atherogenicity. Carcinogenesis studies in rodents and epidemiologic studies in humans suggest a potential role of dietary fibre from coconut kernel in the prevention of colorectal cancer (Hill, 1995).

The anti-diabetic activity of *Momordica charantia* (bitter gourd) was claimed by Teoh *et al.* (2010) and he conducted his experiment in the diabetic induced male Sprague Dawley rats. Zahedani *et al.* (2015) reported a comparative study on the effects of edible oils: rice bran, grape seed, and canola on serum lipid profile and paraoxonase activity in hyperlipidemic rats. The results indicated a significant difference in the grape seed oil group regarding all the measured parameters, except for paraoxonase activity. Moreover, canola oil diet showed a significant hypolipidemic effect on serum Triglyceride and Total Cholesterol and led to a slight improvement in Low Density Lipoprotein-Cholesterol and High Density Lipoprotein-Cholesterol.

In vitro cell culture studies are highly desirable as it provides systems for ready and direct access to cell toxicity mechanisms (Allen *et al.*, 2005). Cell culture is the process by which cells are grown under controlled conditions, generally outside of their natural environment. Common human cell lines used in wet lab experiments are HeLa (derived from cervical cancer cells), HepG2 (human liver carcinoma cell line), HCT15 (colorectal adenocarcinoma) and RAW (murine carcinoma).

Fauser *et al.* (2013) reported that the induction of apoptosis by the medium-chain length fatty acid, lauric acid in colon cancer cells due to induction of oxidative stress. Hepato carcinoma has become one of the major types of cancer with high mortality rate. Lim *et al.* (2014) studied the cytotoxic effect of Virgin Coconut Oil on human Hepatocellular Carcinoma (HepG2) cells and reported that the VCO was effective in reducing cell proliferation. The erythroid leukemic cell lines (K562) were used in the above study.

In-vitro cytotoxicity activity of *Solanum nigrum* extract against Hela cell line and Vero cell line were studied by Patel *et al.* (2009). He claimed that the extract of this plant showed greater activity on HeLa cell line and little activity on Vero cell line and that meant *Solanum nigrum* could be used as anticancer agent. Similarly Ampasavate *et al.* (2010) reported that the extract from fruit plant was a potential source of antileukemic agents.

Real-time PCR (qPCR) applications have become broadly used tools for quantifying gene expression (Kindich *et al.*, 2005). The use of real-time RT-PCR offers several advantages including the relatively small amount of sample required for analysis, the ability to reproduce rapid and accurate data, and the capacity for analyzing more than one gene at a time. deCremoux *et al.* (2003) compared the expression of a number of genes in two different human breast cancer model systems for development of acquired resistance to antiestrogens using a quantitative real-time RT-PCR technique.

Lymphoma is a group of cancers with diverse clinical courses. Diagnosis of the molecular targets involved in the lymphoma cancers, their gene profiling and quantitative gene expression measurements using Real Time PCR will help the effective treatment of cancers (Stahlberg *et al.*, 2005).

Aldose reductase (AR) is the key enzyme in polyol pathway and plays an important role in glucose metabolism. Zhao *et al.* (2012) reported that AR regulates tumor-necrosis-factor-α-induced (TNF-α-induced) iNOS expression in human mesangial cells (HMC) via nuclear factor κB (NFκB) signal pathway. The TNF-α-induced iNOS expression in HMC with different level of AR was measured by Real-time PCR and Western blot.

Hence in the conclusive summary of the available reviewed literature, it is observed that even though the medicinal properties of coconut are highlighted in many aspects, research reports are essential to provide a detailed scientific back ground to support the beneficial effects.

MATERIALS AND METHODS

3. MATERIALS AND METHODS

The study entitled "Molecular docking and validation of medicinal effects of coconut [*Cocos nucifera* L.]" was carried out in two stages viz. *in silico* studies and wet lab studies.

In silico work was carried out at Distributed Information Centre (DIC), Centre for Plant Biotechnology and Molecular Biology (CPBMB), College of Horticulture, Kerala Agricultural University during 2013-2015 with the objective to identify the medicinal properties of coconut compounds by molecular docking studies. The molecular interaction of coconut phyto compounds with different targets involved in major disorders like cancer, alzheimer's, diabetes and hyperlipidemics were studied.

The positive results obtained in the *in silico* study were validated through wet lab analysis. This included cell line studies, response in model animals and gene expression through *qRTPCR*. Since KAU does not have an Institutional Animal Ethics Committee sanction, cell line studies and work with animal model were carried out at Amala Cancer Research Center, Thrissur.

3.1 MATERIALS

3.1.1 Materials used in the *in silico* analysis

3.1.1.1 Work Station and software

For performing *in silico* studies, work station viz. a computer was required in which software was installed for molecular docking studies. Distributed Information Centre (DIC) at KAU maintains work stations with high computing facilities installed with high graphics softwares. The work was carried out on a computer system with Intel i3 processor and 4 GB RAM. The server system with the software ran on Intel i7

processor and 8 GB RAM. All the systems used windows 7 as the operating system. The exclusive and commercial licensed software used for research purpose on molecular docking was Discovery Studio 4.0 developed and distributed by Accelrys, USA. Discovery Studio is a suite of software for simulating small molecule and macromolecule systems. This product suite has a strong academic collaboration programme, supporting scientific research and makes use of a number of software algorithms developed originally in the scientific community, including CHARMM, MODELLER, DELPHI, ZDOCK, DMol3 etc. Discovery Studio provides software applications covering the areas such as simulations, ligand design, pharmacophore modeling, structure-based design, QSAR and ADME.

3.1.1.2 Databases and tools

For literature survey, MEDLINE (Medical Literature Analysis and Retrieval System Online) and PubMed databases, which contain references and abstracts on life sciences and biomedical topics, were accessed. Different ligand databases like Pubchem, Phytochemical databases etc. were used for ligand selection and analysis. Protein Data Bank (PDB) was used for 3D target protein structure retrieval and further studies. Molecular docking analysis was done using CDOCKER protocol in Accelery's Discovery studio tool. CDOCKER is a grid-based molecular docking method that employs CHARMM force fields. CHARMM (Chemistry at HARvard Macromolecular Mechanics) is the name of a widely used set of force fields for molecular dynamics

3.1.1.3 Ligands and protein targets selected

Coconut is enriched with Medium chain fatty acids and polyphenols. Medium chain fatty acids are lauric, capric, caprylic and myristic acids. Polyphenols include caffeic and gallic acids. Both of these were selected as the ligands. The different target proteins of four diseases such as hyperlipidemics, diabetes, alzheimer's and

cancer having crucial role in the disease pathway were identified through literature survey and used in the docking analysis.

3.1.2 Wet lab materials

3.1.2.1 Laboratory chemicals, glass wares and equipments

The chemicals and glass wares used for the work are of good quality (AR grade) and were procured from Sigma Aldrich, Life Technologies, Himedia, SISCO research laboratories 3 (SRL), Invitrogen, Takara Bioindia Pvt.Ltd. and Tarson India Ltd. The list of chemicals and items procured for the work are mentioned in Table 2. Centrifugation was done in high speed centrifuge. UV-VIS Spectrophotometer 119 was used to get the absorbance values for different chemical assays. The other equipments used for the study include Haemocytometer, Incubator, Thermal cycler, Compound microscope and Inverted microscope. Applied Biosystems 7300 Thermal cycler was used for qPCR analysis.

3.1.2.2 Source of animal

Thirty male Sprague dawley rats were collected from Small Animal Breeding Station, Kerala Veterinary and Animal Science University, Mannuthy, Thrissur, weaned for two months and were used as the animal model. All the experiments conducted during the present study had prior permission from the Institutional Animal Ethics Committee (IAEC) and strictly followed the guidelines of Animal Ethics Committee, Government of India.

3.1.2.3 Cell Lines

Cancer cell lines utilized in the study were HepG2, HCT-15 and RAW 264.7. HepG2 is a perpetual cell line, which was derived from hepatocellular carcinoma. HCT-15 stands for human colorectal adenocarcinoma. Raw 264.7 originated from

Table 2: List of chemicals and plastic wares used for wet lab studies

Sl. No.	Product Name	Source
1	HEPES buffer	Himedia Mumbai, Maharashtra
2	Sodium Pyruvate	
3	cDNA direct synthesis kit	Invitrogen, California, USA
4	Fetal Bovine Serum (FBS)	Life Technologies, Invitrogen, California, USA
5	Antibiotics (Penicillin and Streptamycin)	Sigma Aldrich, Missouri, USA
6	Capric acid	
7	Caprylic acid	
8	Lauric acid	
9	Primers	
10	RPMI (Roswell Park Memorial Institute) 1640 Liquid media	
11	Thiazolyl Blue Extrapure (MTT)	SISCO research laboratories (SRL), Mumbai, Maharashtra
12	SYBR premix ex Taq	Takara Bioindia
13	25 cm2 sterile tissue culture flask	
14	96 sterile well plate	
15	6 sterile well plate	

murine leukemic monocyte/macrophage. Cell lines were purchased from NCCS (National Centre for Cell Science), Pune. Cell lines were cultured in RPMI 1640 (Roswell Park Memorial Institute medium) essential media supplemented with 10 per cent Foetal Bovine Serum (FBS), 1ml penicillin (100U) and 100ug/mol streptomycin and kept at 37^0c in an incubator with 5 per cent carbon dioxide and 95 per cent humidity. These cell lines are used for the long term cytotoxicity tests.

3.2 METHODOLOGY

3.2.1 Methods used in the *in silico* molecular docking analysis

3.2.1.1 Identification of ligands

Coconut is a rich source of edible oil having many medicinal properties. Extensive literature survey was done to find out the different phyto compounds in coconut which are effective in improving health. Coconut oil comprises of Medium Chain Fatty Acids such as lauric, capric, caprylic and myristic and polyphenols like gallic and caffeic acids. Medicinal property of coconut is mainly attributed by the presence of these fatty acids.

On the basis of the information obtained from literature, four MCFAs and two polyphenols (mentioned above) were selected as the ligands to analyze the medicinal effects of coconut.

3.2.1.2 Ligand preparation and filtration

The 3D structures of ligands were downloaded from Pubchem database (Plate 1) (maintained by National Centre for Biotechnology Information). These ligands were then cleaned up, calculated 3D coordinates and generated ligand conformations by applying 'prepare ligand protocol' of Discovery Studio 4.0. The steps followed for

performing the work was 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. After preparation, the compounds were filtered based on the molecular properties for predicting their solubility and permeability in drug discovery. The best known filter is the Lipinski's "rule-of-five", which focuses on bioavailability of compounds. The rule states that the ideal compounds should have molecular mass less than 500 daltons, not more than 5 hydrogen bond donors, not more than 10 hydrogen bond acceptors and an octanol-water partition coefficient log P not greater than 5 (Lipinski *et al.*, 2001). Veber's protocol stated that value of polar surface area should not exceed 140 $Å^2$. The filtered compounds were then used for docking analysis.

3.2.1.3 Protein identification and preparation

Modern day diseases like cardiovascular, cancer, diabetes and alzheimer's are largely man-made and iatrogenic in origin. They are affecting the health and well-being of hundreds to millions of people around the world. Detailed research and study about these diseases are essential to minimize their incidence. Excessive quantities of wrongly folded proteins are the cause of diseases like cancer and alzheimer's. Diabetics and hyperlipidemics are caused by the non-healthy food habits.

Extensive literature survey was done about the pathway and mechanisms of the above diseases. The proteins responsible for their occurrence were identified and selected for the interaction study.

The three dimensional structures of target proteins were downloaded from Protein Data Bank (PDB). The structures retrieved from PDB were selected on the basis of the parameters such as those extracted from X ray diffraction process, must contain one or more active sites for binding to ligands, must contain a natural inhibitor to understand its nature of binding and must have a high active site residue

count. Typically a PDB structure file consists of heavy atoms, water molecules and metal ions, and generally has no information on bond orders, topologies or formal atomic charges. It may also have misaligned amide groups, because the X-ray structure analysis cannot usually distinguish between O and NH2. Ionization and tautomeric states are also generally unassigned (Berman, 2008). Considering all these criteria, the 3D structures of proteins were prepared by removing the heteroatoms and cleaned (clean geometry) using Discovery Studio 4 (DS) software (Accelrys Software Inc, USA, 2012) using 'prepare protein' protocol. The steps followed for performing the work was 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. After cleaning, CHARMM force field was applied to the receptor to remove the steric clashes between the atoms in order to get stable conformation. CHARMM is a program for macromolecular dynamics (Brooks *et al.*, 2009).

3.2.1.4 Active site identification

The active site is the region of an enzyme where substrate molecules bind and undergo a chemical reaction. It consists of residues that form temporary bonds with the substrate. The active site is usually a groove or pocket of the enzyme which can be located in a deep tunnel within the enzyme, or between the interfaces of multimeric enzymes. The binding sites of the receptor proteins were predicted based on 'receptor cavity method' using Accelry's Discovery Studio 4.0. For this process, the prepared protein was displayed on the DS 4.0 screen, after which "Receptor ligand interactions" section was accessed to define an active site through PDB site records. In this process, probable binding sites were located looking at their natural ligands i.e. the inhibitors provided along with the crystal structures.

3.2.1.5 Molecular docking

Docking is the virtual screening of a database of compounds and predicting the strongest binders based on various scoring functions. Docking studies of the target proteins were done with natural compounds derived from *Cocos nucifera* to find the preferred orientation and binding affinity of the compounds with each protein using scoring functions.

The factors investigated include the role of a grid representation of a protein ligand interaction, the initial ligand conformation and orientation etc. A representative docking method is used to study the factors namely, CDOCKER, a molecular dynamics (MD) simulated-annealing-based-algorithm. The 'CDOCKER protocol' of docking of ligands with the receptors was performed using Discovery Studio 4 suite to find the interacting compounds of coconut against the target diseases. The steps followed for performing the work was 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.

This method used a grid-based representation of the protein-ligand potential interactions to calculate the binding affinity (Wu *et al.*, 2003). CDOCKER uses soft-core potentials, which are found to be effective in the generation of several random conformations of small organics and macromolecules inside the active site of the target protein. Ligands were docked to the proteins followed by scoring them for their relative strength of interaction to identify candidates for drug development. The final poses were then scored based on the total docking energy, which is composed of intra molecular energy of ligand and the ligand-protein interaction. The parameters observed while docking were ligand energy, complex energy, protein-ligand binding energy, CDOCKER energy, CDOCKER interaction energy, number of hydrogen bonds and the length of hydrogen bonds. Desirable features in molecular docking

included lowest binding energy, shortest hydrogen bond distance, interaction with amino acid available at active sites and minimum deviation in CDOCKER energy and CDOCKER interaction energy.

3.2.1.6 Comparative study

Drugs commonly prescribed for the prevention of above diseases were included in docking analysis for the comparative studies. Informations about the approved drugs were derived from Drug databank (http://www.drugbank.ca/). The dock results of drugs were compared with those obtained for the coconut compounds.

3.2.1.7 Drug likeliness

Drug-likeness is a qualitative concept used in drug design to evaluate how the substance acts like drug with respect to factors like bioavailability. It is estimated from the molecular structure of a compound which is not even synthesized and tested. It has become imperative to design lead compounds which would be easily orally absorbed, easily transported to their targeted site of action, not easily converted into toxic metabolic products and easily eliminated from the body before accumulating in sufficient amounts that may produce adverse side effects. Human intestinal absorption, aqueous solubility, blood brain barrier level, toxicity level, plasma protein binding and CYP2D6 binding are the parameters tested in ADMET analysis.

The sum of the above mentioned properties of absorption, distribution, metabolism, excretion and toxicity is often referred to as ADMET. The prediction of ADMET properties plays a significant role in new drug discovery process (Hire *et al.*, 2012). They are recognized as having a long side therapeutic potential as key determinants of whether a molecule can be successfully developed as a drug or not.

ADMET descriptors in Discovery studio

- Human Intestinal Absorption

It predicts Human intestinal absorption of drug after oral administration. It is defined as per cent absorbed rather than as a ratio of concentration. A well absorbed compound is one that is absorbed at least 90 per cent into the blood stream in humans.

Different prediction levels for Human Intestinal Absorption are:

Score	Prediction
0	Good
1	Moderate
2	Poor
3	Very Poor

- Aqueous Solubility

It predicts the solubility of each compound in water at 25^0C. Aqueous Solubility refers to the distribution and metabolism of a compound in the body. Different prediction levels for Aqueous Solubility are,

<table>
<tr><th>Score</th><th>Prediction</th><th>Drug property</th></tr>
<tr><td>0</td><td>Extremely low</td><td>Can't consider as drug</td></tr>
<tr><td>1</td><td>No/Very low</td><td>May consider as drug</td></tr>
<tr><td>2</td><td>Yes, but still low</td><td rowspan="3">Drug property</td></tr>
<tr><td>3</td><td>Yes, Good</td></tr>
<tr><td>4</td><td>Yes, optimal</td></tr>
<tr><td>5</td><td>No, too soluble</td><td>Drug will be excreted out early</td></tr>
</table>

- Blood Brain Barrier

It predicts the blood brain barrier penetration of a molecule. BBB is defined as the ratio of the concentration of solution on both sides of the membrane (blood-brain). It is relevant while considering the distribution of a compound in the body. Different prediction levels for Blood Brain Barrier are,

Score	**Prediction**
0	Very high
1	High
2	Medium
3	Low
4	Undefined

- Toxicity

Predict the occurrence of dose- dependent human hepatotoxicity. Compounds are classified as either toxic or non-toxic. Different prediction levels for ADMET hepatotoxicity include,

Score	**Prediction**
0 (False)	Non- toxic
1(True)	Toxic

- ADMET CYP2D6 binding

Used to predict the P_{450} 2D6 enzyme inhibition and reports whether or not a compound is likely to be an inhibitor. CYP2D6 enzymes are responsible for the metabolism and elimination of approximately 25 per cent of clinically used drugs. Different prediction levels for ADMET CYP2D6 binding are given below.

Score	Prediction
0 (False)	Non – inhibitor
1 (True)	Inhibitor

- Plasma protein binding

Drug efficiency may be affected by the degree to which it binds to the protein within blood plasma. The less bound a drug is, the more efficiently it can traverse cell membrane or diffuse. Different prediction levels for Plasma protein binding are,

Score	Prediction
0	Binding is <90%
1	Binding is >90%
2	Binding is >95%

3.2.2 Wet lab validation methods

3.2.2.1 *In vivo* methods

3.2.2.1.1 Animals and diets

The experiments were carried out on one month old male Sprague dawley rats weighing about 180 – 200 g, housed in stainless steel cages in a room under controlled conditions (30°C and 80% RH). Thirty rats were randomly assigned for the study. The animals were divided into five groups of which one was kept as control and the other four were treated (n=6). Rats in both control and treated groups were fed with Standard Laboratory Food (SLF), Saidurga Feeds (Bangalore) and water. Different groups were fed with varying doses of lauric acid and coconut oil as detailed below:

Sl. No.	Treatment groups	Diet (In addition to SLF)
1.	LLD	Lauric acid low dose 0.5gm/kg
2.	LHD	Lauric acid high dose 1gm/kg
3.	CO	Coconut oil 2ml/kg
4.	Standard	Atorvastatin 10mg/kg
5.	Control	Only SLF

These doses of fatty acids were fixed based on the standard diet. According to the standard diet 10 per cent of total food (20 g) given to an animal should be fatty acids. So 2 ml of coconut oil was fed to the treated groups. Moreover 50 per cent of coconut oil is lauric acid, based on this fact lauric acid 1 g and 0.5 g doses were fixed. Gain in body weight and water consumption was recorded weekly. The treatment was continued for 30 days. At the end of treatment period, animals were fasted overnight and anesthetized by using chloroform and sacrificed. Blood and liver tissues were collected for various estimations. Liver tissues were stored as such at -20°C. The blood samples were cooled and centrifuged at 5000 rpm for 5 minutes to collect the serum. The collected serum was stored at -20°C for further analysis.

3.2.2.1.2 Lipid profile

2.1. Estimation of cholesterol (Photometric method)

The cholesterol present in the sample originates a coloured complex, according to the following reactions:

Cholesterol esters + $H_2O \xrightarrow{CHE}$ Cholesterol + fatty acids

Cholesterol + $O_2 \xrightarrow{CHOD}$ 4-Cholestenona + H_2O_2

2 H_2O_2+ Phenol + 4-AP $\xrightarrow{POD}$ Quinonimine + $4H_2O$

The intensity of the color formed is proportional to the cholesterol concentration in the sample.

The cholesterol estimation was done by using commercially available Euro Diagnostic Systems test kit (Naito, 1984). Working reagent contained phenol, cholesterol esterase (CHE) cholesterol oxidase (CHOD), peroxidase (POD) and 4-aminoantipyrine (4-AP). Pipetted out 1000 µl of working reagent into a clean test tube. 10 µl of serum sample was added into it, mixed and incubated for 10 minutes at 37°C. Standard was simultaneously analysed by mixing 1000 µl of working reagent and 10 µl of standard cholesterol solution (200 mg/dl). Working reagent 1000 µl alone served as blank. After incubation absorbance was measured at 505 nm in a spectrophotometer. Cholesterol content was estimated as,

Cholesterol mg/dl= OD of Unknown/OD of standard x 200

2.2 Estimation of triglycerides (Photometric method)

The triglyceride estimation was done by using commercially available Euro Diagnostic Systems test kit (Bucolo and David, 1973).

Sample triglycerides incubated with lipoprotein lipase (LPL) liberate glycerol and free fatty acids. Glycerol is converted to glycerol-3-phosphate (G3P) and adenosine-5- diphosphate (ADP) by glycerol Kinase and ATP. Glycerol-3- phosphate (G3P) is then converted by glycerol phosphate dehydrogenase (GPO) to dihydroxyacetone phosphate (DAP) and hydrogen peroxide (H_2O_2). In the last reaction, hydrogen peroxide (H_2O_2) reacts with 4- aminophenazone (4-AP) and p-chlorophenol in presence of peroxidase (POD) to give a red colored dye.

$$\text{Triglycerides} + H_2O \xrightarrow[\text{LPL}]{} \text{Glycerol} + \text{fatty acid}$$

$$\text{Glycerol} + \text{ATP} \xrightarrow{\text{Glycerol Kinase}} \text{G3P} + \text{ADP}$$

$$G3P + 0_2 \xrightarrow{GPO} DAP + H_2O_2$$

$$H_2O_2 + 4\text{- }AP + p\text{-Chlorophenol} \xrightarrow{POD} Quinone + H_2O$$

The intensity of the color formed is proportional to the triglycerides concentration in the sample. Working reagent contained p-chlorophenol, lipoprotein lipase, glycerol kinase, glycerol-3-oxidase, 4-aminophenazone, ATP. Pipetted out 1000 µl of working reagent into a clean test tube. 10 µl of serum sample was added into it. Mixed and incubated for 10 minutes at 37°C. Standard reaction was prepared by mixing 1000 µl of working reagent and 10 µl of standard. Working reagent 1000 µl alone served as blank. After incubation absorbance was measured at 505 nm.

Triglyceride (mg/dl) = OD of Unknown/OD of standard x 200

2.3 Estimation of HDL cholesterol

Direct determination of serum HDLc levels followed the method (Kaplan *et al*., 1984) that depends on the properties of detergent which solubilize only the HDL, so that the HDLc released to react with the cholesterol esterase, cholesterol oxidase and chromogens to give purple colour. The non HDL lipoproteins LDL, VLDL and chylomicrons (lipoproteins) are inhibited from reacting with the enzymes due to the absorption of the detergent on their surfaces. The intensity of the colour formed is proportional to the HDLc concentration in the sample. The test kit of Euro Diagnostic Systems was used for the purpose.

Procedure

The reagent R1 (450 µl) was taken in a test tube and 10 µl of serum sample was added into it. Mixed and incubated for 5 minutes at 37°C. Then 150 µl of R2 reagent was added, mixed and incubated for 5 min. at 37°C. Absorbance was read at 670 nm against the blank.

3.2.2.1.3 Liver enzymes

3.1 Determination of HMG Co-A /Mevalonate Ratio

An indirect method for assessing variation in 3-hydroxy-3-methylglutaryl-coenzyme A reductase (NADPH) activity in liver tissue was followed. 3-Hydroxy-3-methylglu-taryl-CoA and mevalonate concentrations in the tissue homogenate were estimated in terms of absorbance and the ratio between the two is taken as an index of activity of the enzyme, which catalyzes the conversion of 3- hydroxy-3-methylglutaryl-Co A to mevalonate (Rao and Ramakrishnan, 1975).

Reagents used:

1. Saline arsenite solution: 1g of sodium arsenate per liter of physiological saline
2. Dilute perchloric acid (50 ml/ liter)
3. Hydroxylamine hydrochloride reagent (2 mol/liter)

➢ Hydroxylamine hydrochloride reagent for mevalonate: Mixed equal volumes of hydroxylamine hydrochloride reagent and water freshly before use (pH 2.1).

➢ Hydroxylamine reagent for HMG-CoA: Mixed equal volumes of hydroxylamine hydrochloride reagent and sodium hydroxide solution (4.5 mol/liter) freshly before use (pH 5.5).

4. Ferric chloride reagent: Dissolved 5.2 g of trichloro acetic acid and 10 g of ferric chloride in 50 ml of 0.65 mol/liter hydrochloric acid and diluted to 100 ml with the latter.

Procedure:

The liver tissue (1 g) was homogenized in 4 ml of Tris HCL. Mixed equal volumes of fresh tissue homogenate (2 ml) and perchloric acid (2 ml). The mixture was allowed to stand for 5 minute and centrifuged (2000 rpm, 10 min). 1 ml of

filtrate was treated with 0.5 ml of prepared hydroxylamine reagent (add reagents for HMG CoA and mevalonate accordingly), mixed, and after 5 minute added 1.5 ml of ferric chloride reagent to the same tube and shaked well. The absorbance after 10 min incubation at room temperature was measured at 540 nm against a similarly treated saline-arsenate blank.

HMG CoA was determined by using hydroxylamine at pH 5.5 and Mevalonate was estimated by reaction with same reagent, but at pH 2.1. At this pH the, lactone form of mevalonate readily reacts with hydroxylamine to form the hydroxamate. HMG CoA/Mevalonate ratio was then determined.

3.2 Lipoprotein Lipase Assay

Lipoprotein lipase activity was measured using a standardized procedure (Korn, 1959). Lipoprotein lipase in the tissue acts on high triglyceride in the serum (substrate) releasing glycerol and fatty acids. Liberated glycerol is then estimated by chromotropic acid method.

Reagents

- Sorenson's buffer (0.1 M)
- Sodium per iodate (0.05 M)
- Sodium arsenite (0.65 g in 100 ml)
- Freshly prepared chromotropic acid
- TG rich serum

Procedure:

The reaction mixture was prepared by mixing 500 µl of 0.1 M Sorenson's buffer pH 7.4, 200 µl liver homogenate (enzyme source) in Tris HCL and 200 µl TG- rich serum. The volume of reaction mixture was made upto 1 ml with 100 µl distilled water and incubated the samples at 37°C for 1st hour and then 100 µl of

sodium per iodate was added. Another sample with no incubation was allowed to obtain the initial reading. After 5 minutes 100 µl sodium arsenite was added to the mixture and kept it for 10 minutes at room temperature. Then 9 ml of chromotropic acid was added and mixed well. Kept the mixture for 30 minutes in boiling water bath and took OD at 570 nm.

Calculation

From glycerol standard graph, determine concentration of glycerol formed at 0th and at 60th minute time period in nmol.

$$\% \text{ difference nmol (B)} = \frac{\text{Final concentration} - \text{Initial concentration}}{\text{Initial concentration}} \times 100$$

$$\text{Lipase activity} = \frac{\text{B x Differentiation factor}}{(\text{T2} - \text{T1}) \text{ x Volume}}$$

Where, differention factor $= \frac{\text{Final 60} - \text{Initial 0}}{60 \times 50} \times 1$, 60 - Time interval (T_2 -T_1) and 50 - Volume taken for test

Unit = nmol/min/ml or mu/ml

3.3 Sorbitol De-hydrogenase assay

Cells use glucose for energy; however, unused glucose enters the polyol pathway when aldose reductase reduces it to sorbitol. This reaction oxidizes NADPH to NADP+. Sorbitol dehydrogenase can then oxidize sorbitol to fructose, which produces NADH from NAD+. Hexokinase can return the molecule to the glycolysis pathway by phosphorylating fructose to form fructose-6-phosphate. In uncontrolled diabetics that have high blood glucose - more than the glycolysis pathway can handle - the reaction's mass balance ultimately favors the production of sorbitol. Polyol pathway in systems as follows:

Glucose —Aldose reductase (NADPH → $NADP^+$)→ Sorbitol —Sorbitol dehydrogenase (NAD^+ → NADH)→ Fructose

Continuous spectrophotometric rate determination method was followed to assess the rate of sorbitol de-hydrogenase enzyme synthesized.

Reagents used are,

A. 100 mM Tri ethanol amine buffer, pH 7.6 at 25 °C

Prepared 100 ml in deionized water using Tri ethanol amine hydrochloride. Adjusted pH to 7.6 at 25 °C with 5 M NaOH.

B. 1.1 M D- Fructose solution

Prepared 5 ml in deionized water using D (-) fructose

C. 12.8 mM β-Nicotinamide Adenine Dinucleotide

Reduced form solution (β- NADH)

Dissolved the contents of one 10 mg vial of β- NADH reduced form, pre weighed vial, disodium salt in the appropriate volume of deionized water

D. 10 % (w/v) Bovine Serum Albumin (BSA)

Prepared 10 ml in deionized water using Bovine Albumin

E. Sorbitol de-hydrogenase enzyme solution

Prepared a solution containing 70 – 150 unit/ml of sorbitol de-hydrogenase in cold de-ionized water. Stored at 4°C for 1 hr. Immediately before use, diluted to final concentration of 0.55 – 0.75 unit/ml with cold reagent D.

Procedure

- Pipetted (in milli liters) out the following reagents in suitable cuvettes. Volume of reagents used in accessing sorbitol de-hydrogenase are,

	Test (ml)	**Blank (ml)**
Reagent A (Buffer)	2.35	2.35
Reagent B (Fructose)	0.50	0.50
Reagent C (β- NADH)	0.05	0.05

- Mixed by inversion and equilibrium to 25^{0}C. Absorbance was monitored at 340 nm until constant, using a suitably thermostatic spectrophotometer then added,

	Test (ml)	**Blank (ml)**
Reagent E (Enzyme solution	0.10	-
Reagent D (BSA)	-	0.10

- Immediately mixed the reaction mixture by inversion and recorded the decrease in A_{340} nm for approximately 5 minutes.
- Obtained the A_{340} nm/min using the maximum linear rate for the test and blank

Calculations

$$\text{Units/ml enzyme} = \frac{[\,(\Delta A_{340} nm/(Minute\ test)) - (\Delta A_{340} nm/Minute\ blank)\,] \times 3 \times df}{6.22 \times 0.1}$$

3 – Total volume in milli liters

df - Dilution factor

6.22 - Milli molar extinction co-efficient of β- NADH at 340 nm

0.1 – Volume of enzyme in milli liters

$$\text{Units/mg solid} = \frac{\text{Units/(ml enzyme)}}{\text{(mg solid)/(ml enzyme)}}$$

$$\text{Units/ mg protein} = \frac{\text{Units/(ml enzyme)}}{\text{(mg protein)/(ml enzyme)}}$$

One unit will convert 1.0 μmol of D- fructose to D- sorbitol per minute at pH 7.6 at 25^0C.

3.4 Aldose reductase assay

Activation of polyol pathway due to increased aldose reductase (ALDR2) activity has been implicated in the development of diabetic complications. In a hyperglycemic state, the affinity of aldose reductase for glucose rises, causing much sorbitol to accumulate, and using much more NADPH, leaving less NADPH for other processes of cellular metabolism. This change in affinity is what is meant by activation of the polyol pathway. So determination of the rate of aldose reductase enzyme can indicate the presence of hyperglycemic state.

Reagents used for the assay are,

- Lithium sulphate (0.4 mM)
- Mercapta ethanol (5 μmol)
- Glyceraldehyde (10 μmol)
- NADPH (0.10 μmol)

Procedure:

Continuous spectrophotometric rate determination method was followed to assess the rate of Aldose reductase enzyme synthesized. The assay mixture in 1 ml

contained 50 µmol potassium phosphate buffer pH 6.2, 0.4 mmol lithium sulphate, 5µmol 2-mercapita ethanol, 10 µmol DL- glyseraldehyde, 0.1 µmol NAPH and enzyme preparation. One unit was defined as micromoles NADPH oxidized/gHb/min.

The assay mixture was incubated at 37^0C. The change in the absorbance at 340 nm due to NADPH oxidation was recorded (Reddy *et al.*, 2008).

Calculations

$$\text{Units/ml enzyme} = \frac{[(\Delta A_{340} nm/(Minute\ test)) - (\Delta A_{340} nm/Minute\ blank)] \times 3 \times df}{6.022 \times 10^{3} \times 0.1}$$

3 – Total volume in milli liters

df - Dilution factor

6.022×10^{3}- Milli molar extinction co-efficient of β- NADPH at 340 nm

0.1 – Volume of enzyme in milli liters

$$\text{Units/mg solid} = \frac{\text{Units/(ml enzyme)}}{\text{(mg solid)/(ml enzyme)}}$$

$$\text{Units/ mg protein} = \frac{\text{Units/(ml enzyme)}}{\text{(mg protein)/(ml enzyme)}}$$

3.2.2.2 Cell culture studies (*in vitro*) methods

3.2.2.2.1 MTT procedure for long term cytotoxicity assay

The MTT assay is for assessing cell metabolic activity. NAD(P)H-dependent cellular oxidoreductase enzymes may, under defined conditions, reflect the number of viable cells present. These enzymes are capable of reducing the tetrazolium dye

MTT 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide to its insoluble formazan, which has a purple color.

Procedure

- The cells were plated at a density of 100,000 per well in a 6 well plate containing RPMI media and incubated at 37^0C For 24 hrs
- After incubation, cells were treated with the extract at different concentration at 37^0C for 48 hrs
- Culture medium was removed and treated cells were gently washed using autoclaved PBS. Aspirated the PBS
- Then fresh medium was added to each well containing cells
- 100 μl of MTT (5 mg/ml) was added to each well and incubated at 37^0C for 4 hrs
- The test also included a blank containing complete culture medium without cells
- After the incubation, the dark formazan crystals formed were dissolved in 1 ml of solubailisation solution (DMSO) by continuous aspiration and resuspension. The plate was incubated for 15 minutes at 37^0C
- Absorbance of the coloured product was measured at 570 nm
- The cytotoxicity was determined by comparing the percentage death of treated cell population with the untreated control, indicated by their respective absorbance assessed with the MTT assay

3.2.2.1.4 Gene Expression assay

3.2.2.1.4.1 Real Time PCR protocol (qPCR)

A real-time polymerase chain reaction is a laboratory technique of molecular biology based on the polymerase chain reaction (PCR). It monitors the amplification of a targeted DNA molecule during the PCR in real-time, and not at its end point. qPCR was used to analyze the gene expression profile of two enzymes Aldose

reductase and EGFR. For the analysis, the cDNA of both genes were synthesized first. HCT 15 cell lines were treated with different concentration of lauric acid and kept it for 24 hr incubation. Later the cells were harvested and synthesized the cDNA. This cDNA were used as the template for qPCR amplification along with a reference gene β actin. Detailed protocol of cDNA synthesis and qPCR are given below.

4.1.1 Direct cDNA synthesis procedure

For performing Real Time PCR, good quality cDNA was synthesized using SuperScript™ III CellsDirect cDNA Synthesis System. It is an optimized kit for synthesizing first-strand cDNA directly from mammalian cell lysate without first isolating the RNA. Lysis and reverse transcription are performed in the same tube, and the resulting first-strand cDNA was ready to use for further real-time quantitative RT-PCR analysis. The procedure for the cDNA synthesize as per the kit is described below.

Procedure

1. Cell Lysis

- Aspirated the media from each well of the 6 well plate where HCT 15 cell line treated with lauric acid were incubated for 24 hr and washed each well with 1X cold PBS. Then aspirated the PBS.
- Added Resuspension Buffer (100 μl) to each well.
- Incubated the plates on ice for up to 10 minutes. During this period, the plates were tapped periodically and checked the cells under a microscope every 2–3 minutes to see whether they have detached or burst.
- After 10 minutes, gently pippeted out the cells up and down to dislodge the remaining attached cells.

- Estimated the cell density based on the seeding density (10 µl should contain <10,000 cells).
- 10 µl of the cell suspension were transferred to a 0.2-ml thin-walled PCR tubes.

  Control: For the control reaction, added 10 µl of Resuspension Buffer to a PCR tubes and then add 1 µl of Control HeLa Total RNA.
- Added 1 µl of RNaseOUT™ (40 U/µl) to the PCR tubes.
- Then tubes were transferred to an incubator or thermal cycler preheated to 75°C and incubated for 10 minutes.

  Control: For the control reaction, incubate for 3 minutes.

2. DNase I Digestion

- Placed the above tubes on ice, and added the following:

Component	**Amount**
DNase I, Amplification Grade (1 U/µl)	5 µl
10X DNase I Buffer	1.6 µl

- The reagents were mixed gently pipetting up and down and spinned briefly to collect the contents. The tubes were incubated for 5 minutes at room temperature.
- The tubes were spinned briefly and added 1.2 µl of 25 mM EDTA to each well on ice. Mixed by gently pipetting up and down and again spinned briefly to collect the contents.
- Samples were incubated at 70°C for 5 minutes. Then spinned briefly and proceeded to First-Strand cDNA Synthesis.

3. First-Strand cDNA Synthesis

- Placed each tube from DNase I Digestion, on ice, and added the following reagents:

Component	**Amount**
Oligo(dT)20 (50 mM)	2 μl
10 mM dNTP Mix	1 μl

- Then mixed by gently pipetting up and down and spinned the tube briefly to collect the contents.
- Incubated the tube at 70°C for 5 minutes. Again spinned the tube briefly to collect the contents.
- Placed the tubes on ice for 2 minutes, and then added the following reagents:

Component	**Amount**
5X RT Buffer	6 μl
RNaseOUT™ (40 U/μl)	1 μl
SuperScript™ III RT (200 U/μl)*	1 μl
0.1 M DTT	1 μl

- For negative RT controls, used 1 μl of sterile, distilled water instead of SuperScript™ III RT
- Mixed by gently pipetting up and down and spinned the tube briefly to collect the contents.
- Transfered the tube to a thermal cycler preheated to 50°C. Incubated it for 50 minutes.
- Inactivated the reaction at 85°C for 5 minutes. Added 1 μl of RNase H (2 U/μl) to each tube and incubated at 37°C for 20 minutes.
- Chilled the reaction on ice. Stored the single-stranded cDNA at –20°C.

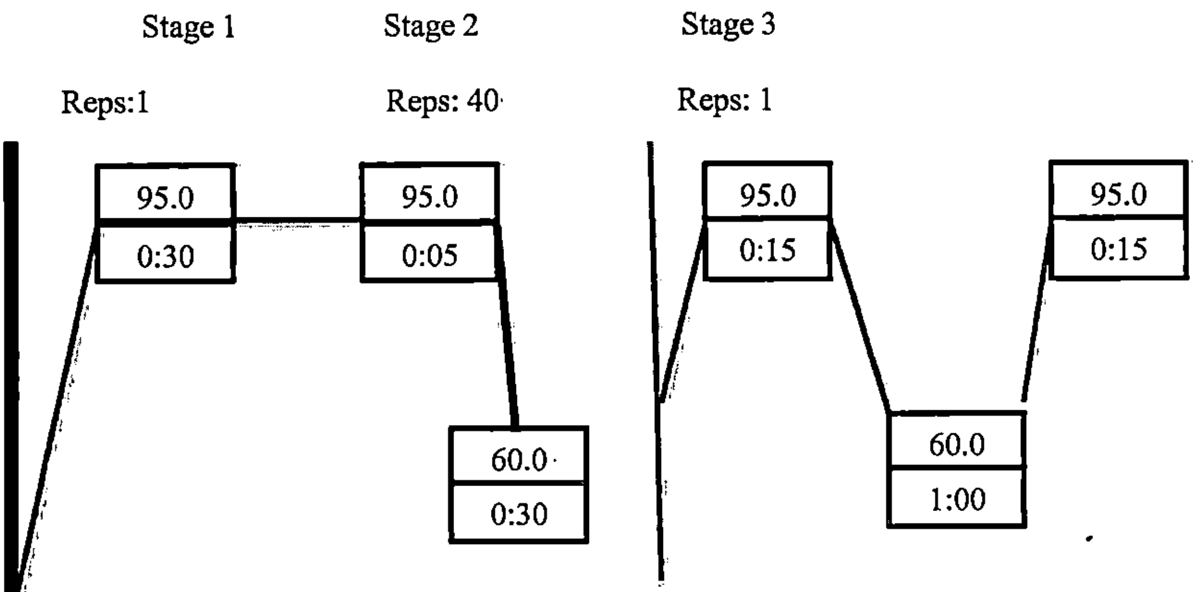
4.1.2 *qRTPCR* protocol

The amplication was carried out in a Thermal Cycler, Applied Biosystems 7300. The *Real Time PCR* was performed to study the gene expression profile of two enzymes Aldose reductase and EGFR. Data normalisation in real-time RT-PCR is a further major step in gene quantification analysis (Bustin, 2002 and Pfaffl, 2001). The reliability of any relative qRT-PCR experiment can be improved by including an invariant endogenous control (reference gene) in the assay to correct for sample to sample variations in qRT-PCR efficiency and errors in sample quantification. β actin gene was used as the reference gene in the present analysis.

PCR amplication was performed in a 25 μl reaction mixture which consisted of,

(a) SYBER *Premix Ex Taq* (2X)	- 12.5 μl
(b) PCR Forward primer	- 1 μl
(c) PCR Reverse primer	- 1 μl
(d) ROX Reference Dye	- 0.5 μl
(e) Template cDNA	- 5 μl
(f) Sterile distilled water	- 5 μl
Total volume	- 25 μl

The reverse and forward primers for EGFR and Aldose reductase genes were used in the respective samples. Software used in the qRTPCR analysis was 7300 System SDS Software. The amplification was carried out with the following programme:



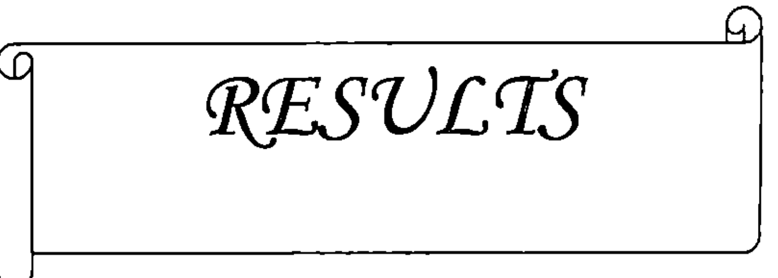
qRT-PCR standard protocol

- Stage 1: Initial denaturation Reps: 1 95 °C, 30 sec
- Stage 2: PCR Reps: 40 95 °C, 5 sec 60 °C, 30 sec
- Stage 3: Dissociation stage

  Reps: 1

  - 95 °C, 15 sec
  - 60 °C, 1 min
  - 95 °C, 15 sec

Ct values obtained from the melting curve of qPCR were then used to calculate the ΔCt values and ΔΔCt values. The fold change (ΔΔCt) in the target gene relative to the endogenous control (β actin) gene is, **ΔΔCt = ΔCt (Test) - ΔCt (Control) Where, ΔCt = Ct (Target) - Ct (Reference)**


RESULTS

4. RESULTS

The observations recorded and the results obtained in the study "Molecular docking and validation of medicinal effects of coconut [*Cocos nucifera* L.]" are presented in this chapter.

4.1 MOLECULAR DOCKING THROUGH *IN SILICO* ANALYSIS

4.1.1 Ligand preparation

The natural compounds selected as ligands from coconut include lauric acid, capric acid, caprylic acid, myristic acid, gallic acid and caffeic acid. The ligands were prepared using "prepare ligand" protocol in Discovery Studio 4.0. Prepared ligands were then filtered using Veber and Lipinski's rule of five and results are provided in Table 3. Among the six, five ligands passed the Veber and Lipinski's rule and are expected to be active compounds for oral administration. Myristic acid failed the Veber and Lipinski's rule since its logP value exceeded 6.

4.1.2 Protein target selection and preparation

Through extensive literature survey the different target proteins involved in the metabolic pathway of diseases like cancer, alzheimer's, diabetes and hyperlipidemics were identified. These diseases are non-pathogenic in nature. The targets identified for each disorder along with their references are provided in Table 4. For carcinoma, seven targets were identified while it was three for alzheimer's, two for diabetes and two for hyperlipidemics. Thus all together fourteen targets were selected for the study.

Cancer also known as a malignant tumor is a group of diseases which causes the abnormal cell growth with the potential to invade or spread to different parts of the body. Protein targets identified for carcinoma in the present *in silico* analysis were EGFR, CDK, DHFR, TS, VEGFR, ER and Bcl-xl. Epidermal growth factor receptor (EGFR) is a member of the receptor tyrosine kinase family.

Table 3: Drug likeliness properties of ligands as per Veber and Lipinski's rule (Lipinski *et al.*, 2001)

Sl. No.	Coconut compounds (Ligands)	Veber and Lipinski's parameters for screening*				
		Mol. Wt. (<500 daltons)	H bond acceptor (<10)	H bond donor (<5)	logP (≤5)	TPSA (<140)
1	Capric acid	172.268	2	1	4.02	37.29
2	Caprylic acid	144.214	2	1	3.01	37.29
3	Lauric acid	200.322	2	1	5.03	37.29
4	Myristic acid	228.376	2	1	6.04	37.29
5	Caffeic acid	207.203	4	1	0.42	58.59
6	Gallic acid	143.203	2	1	1.26	40.12

*Desirable values are provided in the parentheses

Table 4: Details of different protein targets identified for the diseases

Disease	Protein targets	PDB ID	Reference
Carcinoma	Epidermal Growth Factor Receptor (EGFR)	1M17	Normanno *et al.*, 2006 Forastiere and Burtness, 2007
	Cyclin dependent kinase (CDK)	1GII	Loyer *et al.*, 2005 Sanghani *et al.*, 2012
	Dihydrofolate reductase (DHFR)	1DRF	Gond *et al.*, 2013
	Thymidylate synthase (TS)	1I00	Srivastava *et al.*, 2010
	Vascular Endothelial Growth Factor Receptor 2 kinase (VEGFR)	3HNG	Asthana *et al.*, 2014
	Estrogen receptor (ER)	1ERR	Lund, 2005
	Bcl-xl	3ZK6	Kalenkiewicz *et al.*, 2015
Alzhimers	Amyloid precursor protein	3KTM	Priller *et al.*, 2006
	Beta secretase 1	1W51	Gupta *et al.*, 2010
	Beta secretase 2	2EWY	Gupta *et al.*, 2010
Diabetes	Aldose reductase	1IEI	Akhila *et al.*, 2012
	Dipeptidyl peptidase IV	3F8S	Anitha *et al.*, 2013
Hyperlipidemics	HMG Co A reductase	1DQ8	Chandran *et al.*, 2011
	Cholesterol esterase	1F6W	Sivashanmugam *et al.*, 2013

Amplification of the EGFR gene and mutations of the EGFR tyrosine kinase domain have been recently demonstrated to occur in carcinoma patients. These mutations have been associated with a number of cancers, including lung cancer, anal cancer and Glioblasto mamultiforme (GBM- brain tumor) (Normanno *et al.*, 2006). Cyclin-dependent kinases (CDKs) are a family of protein kinases first discovered for their role in regulating the cell cycle. Mutational changes in these molecules lead to the perturbed cell cycle leading to uncontrolled cellular proliferation or cell death. In humans, mutations in cyclin dependent kinase 2 are responsible for nearly 50 per cent of cancers (Sanghani *et al.*, 2012).

Dihydrofolate reductase (DHFR) enzyme is encoded by the *DHFR* gene. DHFR is responsible for the levels of tetrahydrofolate in a cell, and the inhibition of DHFR can limit the growth and proliferation of cells that are characteristic of cancer (Gond *et al.*, 2013). DNA damage or deletion occurs on a daily basis as a result of both endogenous and environmental factor. Thymidylate synthase (TS) plays a crucial role in the early stages of DNA biosynthesis. Synthesis and insertion of healthy DNA is vital for normal body functions and avoidance of cancerous activity. For this reason, TS has become an important target for cancer treatment by means of chemotherapy (Srivastava *et al.*, 2010).

Vascular endothelial growth factor receptor (VEGFR) is a part of the metabolic system that restores the oxygen supply to tissues when blood circulation is inadequate. When VEGFR is overexpressed, it can contribute to disease. Solid cancers cannot grow beyond a limited size without an adequate blood supply, cancers that can express VEGFR are able to grow and metastasize. Estrogen receptors are over-expressed in around 70 per cent of breast cancer cases, referred to as "ER-positive" (Lund, 2005). Binding of estrogen to the ER stimulates proliferation of mammary cells, with the resulting increase in cell division and DNA replication, leading to mutations. The result is the disruption of cell cycle, apoptosis and DNA repair, and tumour formation. B-cell lymphoma-extra-large is a trans membrane

molecule in the mitochondria. The prosurvival BCL-2 family protein Bcl-xl is often overexpressed in solid tumors and renders malignant tumor cells resistant to anticancer therapeutics (Kalenkiewicz *et al.,* 2015).

Alzheimer's disease (AD) accounts for 60 per cent to 70 per cent cases of dementia. Protein targets identified for alzheimer's used in the *in silico* analysis are APP and BACE 1 & 2. The disease process is associated with formation of plaques and tangles in the brain. Amyloid precursor protein (APP) is best known as the precursor molecule whose proteolysis generates beta amyloid (Aβ), a 37 to 49 amino acid peptide whose amyloid fibrillar form is the primary component of amyloid plaques found in the brains of Alzheimer's disease patients (Priller *et al.*, 2006). Extracellular cleavage of APP is initiated by BACE1 gene. Levels of this enzyme have been shown to be elevated more in the common late-onset sporadic Alzheimer's. BACE2 is a close homolog of BACE1 with no reported APP cleavage *in vivo* (Gupta *et al.,* 2010). Human BACE shows a great promise as a potential therapeutic target for Alzheimer's disease.

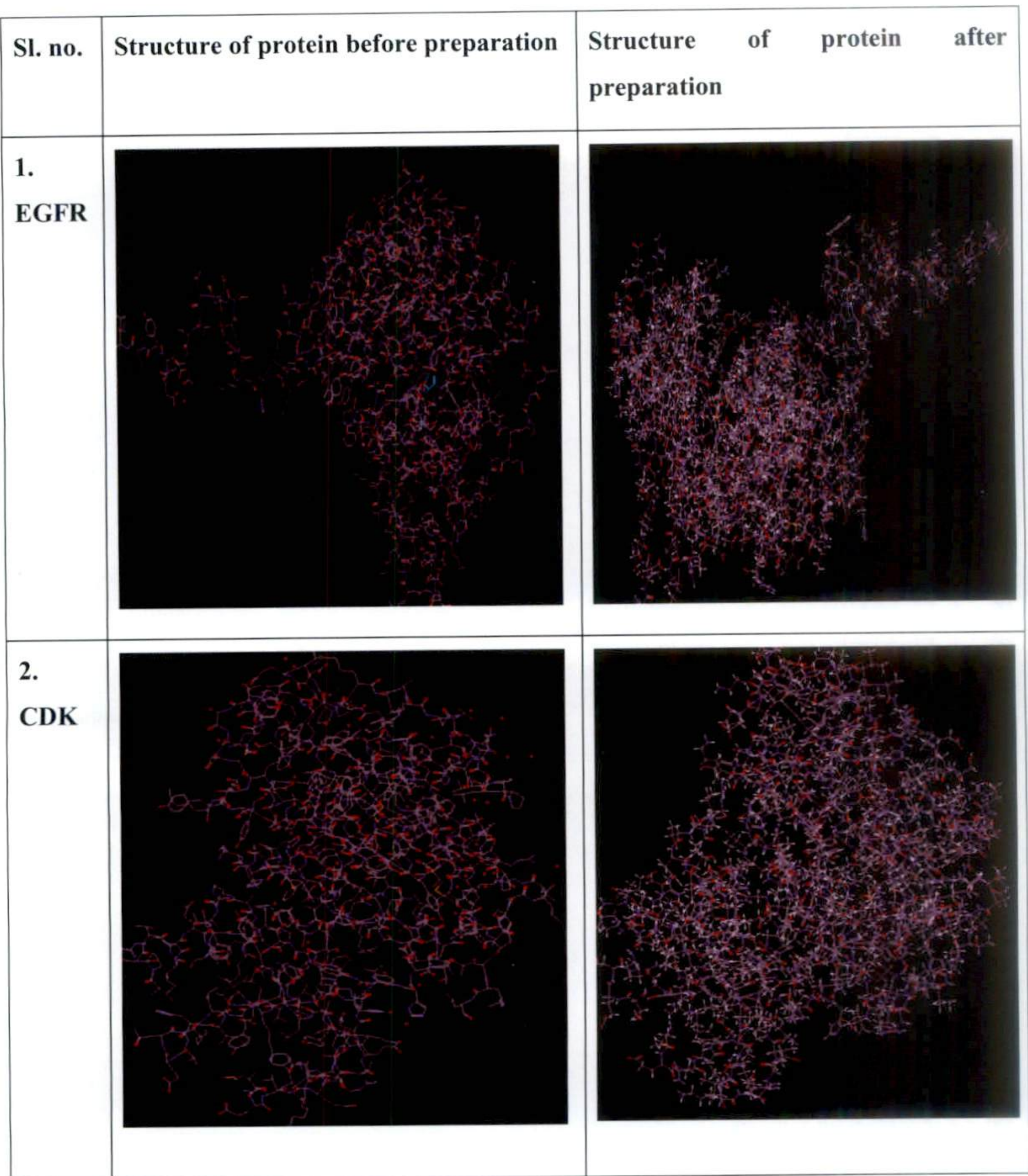
Diabetes, often referred as diabetes mellitus, describes a group of metabolic diseases in which the person has high blood glucose, either because insulin production is inadequate, or because the body's cells do not respond properly to insulin. Aldose reductase catalyzes the NADPH-dependent conversion of glucose to sorbitol, the first step in polyol pathway of glucose metabolism. In response to the chronic hyperglycemia found in diabetics, glucose flux through the polyol pathway is significantly increased. Glucose concentrations are often elevated in diabetes and aldose reductase has long been believed to be responsible for diabetic complications involving a number of organs (Akhila *et al.*, 2012). DPP-IV is a serine protease cleaving the N-terminal dipeptide with a preference for L-proline or L-alanine at the penultimate position 7. Inhibition of DPP-IV increases the level of circulating GLP-1 and thus increases insulin secretion, which can ameliorate hyperglycaemia in type2

diabetes (Anitha *et al.*, 2013). In the present *in silico* analysis, aldose reductase and DPP-IV proteins were identified as the targets for diabetes.

Hyperlipidemia involves abnormally elevated levels of any or all lipids and/or lipoproteins in the blood. HMG-CoA reductase is the rate-controlling enzyme of the mevalonate pathway, the metabolic pathway that produces cholesterol and other isoprenoids. The reaction catalyzed by HMG-CoA reductase is the rate-limiting step in cholesterol synthesis, this enzyme represents the sole major drug target for contemporary cholesterol-lowering drugs in humans (Chandran *et al.*, 2011). In enzymology, Cholesterol esterase also called as sterol esterase is an enzyme that catalyzes the hydrolysis of sterol esters into their component sterols and fatty acids. The two substrates of this enzyme are steryl ester and H_2O, whereas its two products are sterol and fatty acid. It is responsible for the hydrolysis of various substrates including dietary cholesterol esters, fat-soluble vitamins, triglycerides, and phospholipids (Sivashanmugam *et al.*, 2013).

The three dimensional structures of these proteins were downloaded from PDB data base and were then prepared using 'prepare protein' protocol in Discovery studio. According to this protocol protein structures with more than one chain present, like chain A, chain B etc., chain A alone was used for protein preparation. The other chains along with ligands and water molecules were removed from the protein.

All protein structures were then cleaned by correcting geometries, inserting missing loops, grafting loops, managing conformers and modifying protonation of termini and ionizable side chains. This was followed by energy minimization of the structure to find out stable conformation using CHARMM force field. Structure of selected proteins after preparation is provided in plate 1.



Sl. no.	Structure of protein before preparation	Structure of protein after preparation
1. EGFR		
2. CDK		

Plate 1: 3D structures of selected proteins identified for carcinoma before and after preparation using Discovery studio

4.1.3 Active site prediction and molecular docking of protein targets identified for carcinoma

4.1.3.1 Selection of active sites

The active site is usually a groove or pocket of the enzyme which can be located in a deep tunnel within the enzyme, where substrate molecules bind and undergo chemical reactions. The active site consists of residues that form temporary bonds with the substrate (binding site) and residues that catalyze a reaction of that substrate (catalytic site). A protein usually contains more than one active site. So it is important to find the active site in which the ligands possess an effective interaction. It depends on the number of hydrogen bonds formed between the ligands and the target. Table 5 depicts the no of active sites identified for each cancer target and the one used in the docking analysis. The number of active sites ranged between one and four for the different targets. The possible amino acids involved in the hydrogen bond formation which were obtained from PDB site recorder are also given. More than thirty amino acids were identified in the active site of protein, DHFR whereas only two were identified in case of VEGFR. The number of amino acids varied according to proteins and the strength of interactions.

4.1.3.2 Molecular docking

Molecular docking was performed with the targets identified for each disorder and the ligands selected. The ligands included the five phyto compounds in coconut and nine commercial drugs approved for carcinoma. The details of commercial drugs used in the study are provided in Table 6. The results obtained for each target are described here under.

Table 5: Details of active site and PDB ID of protein targets involved in carcinoma

Sl. no.	Protein	PDB ID	No of active sites	Active sites selected	Amino acid residues in selected active site
1	Epidermal Growth Factor Receptor (EGFR)	1M17	1	Active site 1	Tyr740, Leu838, Glu673 and Ala674, Met 769.
2	Cyclin dependent kinase (CDK)	1GII	1	Active site 1	Gln85, Asn132, Asp86, Asp145, Gln131, Phe80 , Val18, Glu12 and Gly11
3	Dihydrofolate reductase (DHFR)	1DRF	3	Active site 3	Ile7, Val8, Ala9, Ile16, Gly20, Asp21, Leu22, Trp24, Pro26, Leu27, Arg28, Glu30, Phe31, Arg32, Tyr33, Phe34, Gln35, Arg36, Met37, Thr38, Val50, Met52, Thr56, Ser59, Ile60, Pro61, Asn64, Arg65, Leu67, Arg70, Asn72, Val115, Gly116 and Tyr121
4	Thymidylate synthase (TS)	1I00	4	Active site 1	Arg50, Phe80, Leu108, Asn112, Leu192, Asp218, Gly220, Leu221, Gly222, Phe225 and Tyr258
5	Vascular Endothelial Growth Factor Receptor 2 kinase (VEGFR)	3HNG	2	Active site 1	Cys912, Glu878
6	Estrogen receptor (ER)	1ERR	2	Active site 1	Asp351, Glu353, Arg394, Phe404, His524
7	Bcl-xl	3ZK6	2	Active site 1	Arg139, Leu130, Phe97, Gly138, Arg102

Table 6: Details of FDA approved drugs in use for cancer

Sl. no.	Drugs	Drug bank accession no.	Drug specific protein targets	Manufacturing company	Side effects of drugs
1	Gefitinib	DB00317	EGFR (1M17)	Astrazenecauk	Diarrhea
2	Letrozole	DB01006		Novartis, Mylan pharmaceuticals etc.	Hot flashes, arthralgia (joint pain), and fatigue
3	Ponatinib	DB08901	CDK (1GII)	ARIAD Pharmaceuticals	Increased number of blood clots
4	Methotrexate	DB00563	DHFR (1DRF)	Mylan, Roxane laboratories etc.	Mucositis, hepatic toxicity, thrombocytopenia, alopecia
5	Fluorouracil	DB00544	TS (1I00)	Elorac, Taro pharmaceuticals etc.	Nausea, vomiting, reduced white cell count
6	Raltitrexed	DB00293		AstraZeneca Company	Hair loss or thinning, Joint pain, Muscle cramps, Swollen hands
7	Sorafenib	DB00398	VEGFR (3HNG)	Bayer healthcare	Diarrhea, Rash, Pruritus (itchiness), Erythema
8	Sunitinib	DB01268		Pfizer	Fatigue, diarrhea, nausea, anorexia, hypertension, a yellow skin discoloration
9	Tamoxifen	DB00675	ERR	Rosemont group, Astrazeneca pharmaceuticals	Vomiting, constipation, increased thirst or urination, muscle weakness, bone pain

4.1.3.2.1 Interaction of coconut phyto compounds with the protein target Epidermal Growth Factor Receptor (EGFR) identified for carcinoma

The interaction between the phyto compounds identified in coconut with the target EGFR identified for carcinoma in comparison with commercial drug is provided in Table 7. Phyto compounds in coconut recorded a binding energy ranging between -78.91 and -145.8 kcal/mol. Caffeic acid recorded the lowest binding energy. However the variation between CDOCKER energy and CDOCKER interaction energy was high for caffeic acid and the interacting amnoacid residue did not match with the one in the active site. Considering the favourable parameters, lauric acid was observed to have better interactions with EGFR and was found far superior to the commercial drugs available in the market. The binding energy of lauric acid recorded was -102.75 kcal/mol with hydrogen bond distance of 2.1 A^0 and the difference in CDOCKER energy and CDOCKER interaction energy was 4.32 kcal/mol. Also its binding was with MET at 769th position in the identified active site. The commercial drugs (Gefitinib and Lerozole) also had the same interaction with active site but with poor docking scores.

4.1.3.2.2 Interaction of coconut phyto compounds with the protein target Cyclin Dependent Kinase (CDK) identified for carcinoma

The different interactions obtained when the coconut phytocompounds and target specific drug were docked with the protein target CDK are summarised in Table 8. Among the interactions, the least binding energy was recorded for capric acid (-113.86 kcal/mol) compared to other ligands. The CDOCKER energy and CDOCKER interaction energy of capric acid were also high with minimum energy difference . Also this compound was interacting with the protein through the amino acid ASN in 132th (Plate 2) position which matched with the active site residue. Next

Table 7: Dock scores of coconut compounds and the commercial drugs against the protein target EGFR identified for cancer

Protein (PDB ID)	Ligands	(-) CDOCKER energy (kcal/mol)	(-) CDOCKER interaction energy (kcal/mol)	H bond amino acid residues	H bond distance (A°)	Binding energy (kcal/mol)
EGFR (1M17)	*1. Coconut compounds*					
	Caffeic acid	20.43	33.56	ARG752	2.47	-145.8
	Lauric acid	30.47	34.24	MET769*	2.10	-102.75
	Capric acid	30.05	28.84	MET769*	2.31	-95.48
	Gallic acid	29.95	27.39	MET769* THR766 THR766	2.0 1.98 2.24	-80.58
	Caprylic acid	28.19	27.13	MET769*	2.21	-78.91
	2. Commercial drugs					
	Gefitinib	19.48	35.21	MET769*	2.01	-60.76
	Letrozole	-9.40	23.83	MET769*	2.48	-30.10

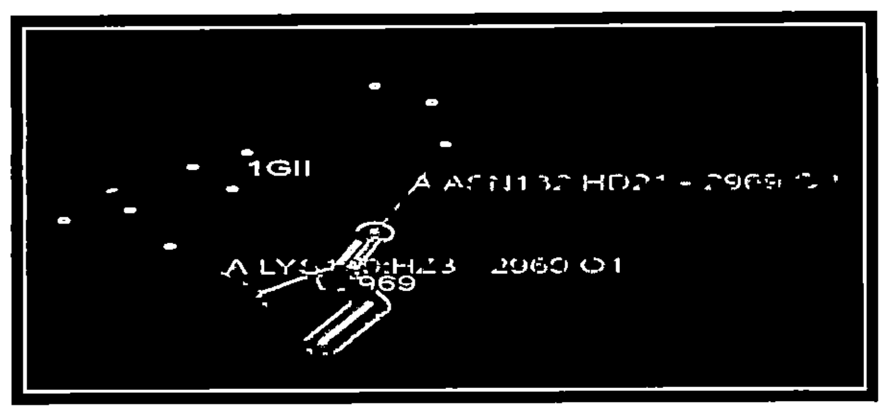
*Lead aminoacid residues in the active site

Table 8: Dock scores of coconut compounds and the commercial drugs against protein target CDK identified for cancer

Protein (PDB ID)	Ligands	(-) CDOCKER energy (kcal/mol)	(-) CDOCKER interaction energy (kcal/mol)	H bond amino acid residues	H bond distance (A°)	Binding energy (kcal/mol
CDK (1GII)	*1. Coconut compounds*					
	Capric acid	40.10	39.44	LYS129 ASN132*	1.89 2.25	-113.86
	Caffeic acid	25.24	34.59	LYS129	2.15	-111.53
	Caprylic acid	32.30	30.86	LYS129 LYS129	2.14 2.04	-109.52
	Lauric acid	39.5	39.87	LYS129 ASN132*	1.87 2.44	-108.11
	Gallic acid	28.90	26.39	ASP145* VAL83	2.39 2.27	-3.07
	2. Commercial drugs					
	Ponatinib	13.03	57.61	LEU298	2.1	-145.11

*Lead aminoacid residues in the active sites

Plate 2: Hydrogen bond interactions between capric acid and CDK



to capric acid, caffeic acid (-111.53 kcal/mol) and lauric acid (-108.11 kcal/mol) recorded the least binding energies. The poor interaction was recorded by gallic acid with a binding energy of -3.07 kcal/mol. The drug, ponatinib scored better binding energy (-145.11 kcal/ mol) than coconut compounds. However the interactions was not superior since the energy difference between CDOCKER energy and CDOCKER interacton energy was too high (44 kcal/ mol) compared to capric acid.

4.1.3.2.3 Interaction of coconut phyto compounds with the protein target Dyhro Folate Reductase receptor (DHFR) identified for carcinoma

Table 9 summarizes the details about binding energy, CDOCKER energy, CDOCKER interaction energy and hydrogen bond interaction obtained when the coconut compounds and synthetic drugs were docked with the protein target DHFR identified for carcinoma. From the table it is clear that lauric acid scored the least binding energy with a value of -185.42 kcal/ mol. Also lauric acid scored 41.68 kcal/mol of CDOCKER energy and 43.35 kcal/mol of CDOCKER interaction energy. The lead amino acids identified for the interaction between DHFR and phyto compounds were ARG70 and THR56. Lauric acid was interacting with the amino acid ARG 70 of DHFR with a bond length of 1.9 A^{0} (Plate 3). Among the phyto compounds lauric acid interacted well with the protein. The synthetic drug, Methotrexate scored a binding energy of -313.2 kcal/ mol. The CDOCKER energy and CDOCKER interaction energy were high for the drug with three hydrogen bonds matching with the active site residue.

Table 9: Dock scores of coconut compounds and commercial drugs against the protein target DHFR identified for carcinoma

Protein (PDB ID)	Ligands	(-) CDOCKER energy (kcal/mol)	(-) CDOCKER interaction energy (kcal/mol)	H bond amino acid residues	H bond distance (A°)	Binding energy (kcal/mol)
DHFR (1DRF)	*1. Coconut compounds*					
	Lauric acid	41.68	43.35	ARG70*	1.9	-185.4
	Caprylic acid	30.22	29.75	THR56* GLY30	1.9 2.10	-81.83
	Caffeic acid	30.45	31.63	THR56* GLY30	1.9 2.2	-71.5
	Capric acid	32.73	32.58	THR56*	1.9	-69.7
	Gallic acid	22.33	19.54	ALA9	2.21	-11.9
	2. Commercial drugs					
	Methotrexate	61.53	71.40	ARG70* ARG70* ARG70* ASN64	2.0 2.1 2.0 1.9	-313.2

*Lead aminoacid residues in the active site

4.1.3.2.4 Interaction of coconut phyto compounds with the protein target Thymidylate Synthase (TS) identified for carcinoma

Details about the different dock scores and hydrogen bond interaction obtained upon docking the coconut phyto compounds and drugs with the target TS is summarized in Table 10. Almost all the ligands possessed a very good interaction with the TS target. Among phyto compounds lauric scored better results. The least binding energy (-213.1 kcal/mol), highest CDOCKER energy and CDOCKER interaction energy and also three hydrogen bonds with two having amino acids similar to active site residues were obtained for lauric acid (Plate 3). Compared to the phyto compounds, the synthetic drug, Raltitrexed scored superior results in all the aspects of interaction. It recorded a binding energy of -448.42 kcal/ mol. The lead amino acid residue identified for the TS targets was ARG in 50th position.

4.1.3.2.5 Interaction of coconut phyto compounds with the protein target Vascular Endothelial Growth Factor (VEGFR) identified for carcinoma

The interactions obtained for coconut phyto compounds when docked with the protein target VEGFR identified for carcinoma in comparison with the synthetic drugs are summarized in Table 11. Phyto compounds in coconut, recorded a binding energy ranging between -109.55 and -60.65 kcal/mol. The least binding energy was recorded by caffeic acid and the highest by gallic acid. The values of CDOCKER energy and CDOCKER interaction energy of caffeic acid were 25.73 and 38.9 kcal/ mol respectively. Next to caffeic acid, lauric acid scored the least binding energy (-92.21 kcal/mol). Also, compared to caffeic acid CDOCKER energy (43.85 kcal/ mol) and CDOCKER interaction energy (42.62 kcal/ mol) of lauric acid was good. Lauric acid had two hydrogen bond interactions with the lead amino acid CYS in 912th position with bond lengths of 2.2 A° and 2.4 A°.

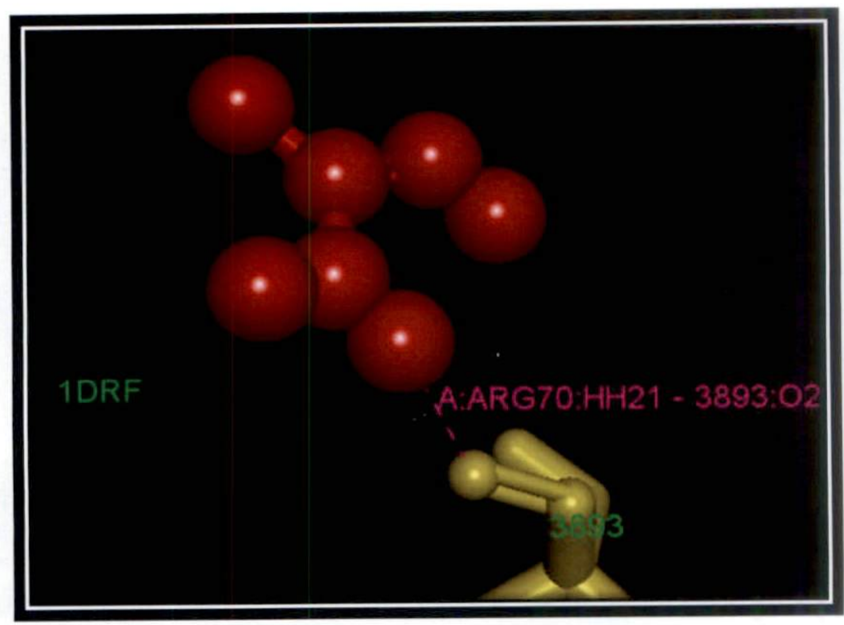


Plate 3: Hydrogen bond interaction between lauric acid and DHFR

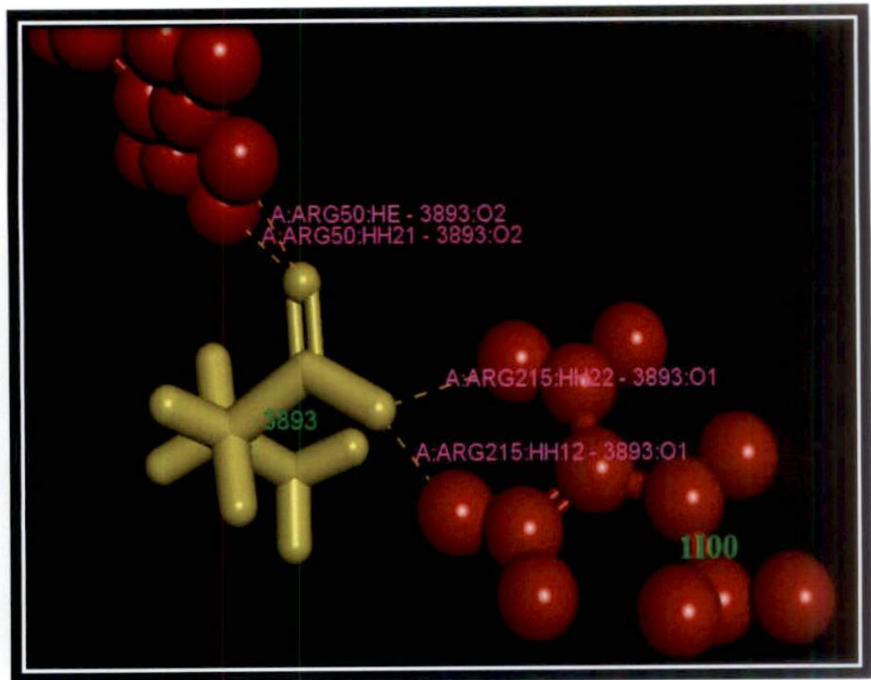


Plate 4: Hydrogen bond interaction between lauric acid and TS

Table 10: Dock scores of coconut compounds and commercial drugs against the target TS identified for carcinoma

Protein (PDB ID)	Ligands	(-) CDOCKER energy (kcal/mol)	(-) CDOCKER interaction energy (kcal/mol)	H bond amino acid residues	H bond distance (A°)	Binding energy (kcal/mol)
TS (1I00)	1. *Coconut compounds*					
	Lauric acid	47.36	45.21	ARG50* ARG50* ARG215	2.15 2.01 1.9	-213.1
	Caffeic acid	30.67	43.39	ARG50* ARG 215 ASP218*	2.11 2.23 2.13	-208.7
	Capric acid	44.01	43.76	ARG50* ARG50* ARG215	2.15 2.31 2.03	-199.3
	Gallic acid	36.87	34.12	ARG50* ARG50*	2.4 1.9	-197.8
	Caprylic acid	39.92	38.42	ARG50* ARG50* ARG215	2.15 2.12 1.98	-193.3
	2. *Commercial drugs*					
	Raltitrexed	66.97	72.88	ARG50* SER216 ASP218*	2.3 2.09 2.2	-448.42
	Fluorouracil	20.57	17.39	HIS196 ASN226	2.0 2.3	-45.56

*Lead aminoacid residues in the active sites

Table 11: Dock scores of coconut compounds and commercial drugs against the target VEGFR identified for carcinoma

Protein (PDB ID)	Ligands	(-) CDOCKER energy (kcal/mol)	(-) CDOCKER interaction energy (kcal/mol)	H bond amino acid residues	H bond distance (A°)	Binding energy (kcal/mol)
VEGFR (3HNG)	*1. Coconut compounds*					
	Caffeic acid	25.73	38.91	LYS861 CYS912*	2.38 2.27	-109.55
	Lauric acid	43.85	42.62	CYS9 12* CYS912 *	2.28 2.45	-92.21
	Capric acid	38.59	38.10	CYS9 12* CYS912 *	2.2 2.43	-81.93
	Caprylic acid	33.09	31.98	CYS912*	2.22	-72.66
	Gallic acid	34.83	33.64	GLU878* GLU878*	2.04 2.02	-60.65
	2. Commercial drugs					
	Sorafenib	42.25	65.44	GLU878* GLU878* CYS912* CYS912*	2.18 2.04 2.1 2.24	-82.37
	Sunitinib	8.741	56.64	-	-	-60.67

*Lead amino acid residues in the active sites

Considering the favorable parameters, lauric acid was observed to have better interactions with VEGFR. While in case of synthetic drugs, Sunitinib had a very poor interaction with the protein whereas Sorafenib possessed four hydrogen bonds similar to active site residue. But the difference in CDOCKER energy and CDOCKER interaction energy of the drug was too high to have an effective interaction with the protein VEGFR.

4.1.3.2.6 Interaction of coconut phyto compounds with the protein target Estrogen Receptor (ER) identified for carcinoma

CDOCKER energy, CDOCKER interaction energy, binding energy and hydrogen bond interaction obtained from the docking analysis between coconut phyto compounds and commercial drugs against the target ER for carcinoma are summarized in Table 12.

Phyto compounds showing binding energy varies from -54 to -115.7 kcal/mol. CDOCKER energy, CDOCKER interaction energy and binding energy of lauric acid found optimum compared to other phyto compounds. The least binding energy was reported for lauric with a value -110.74 kcal/mol. Also the value of CDOCKER energy of lauric acid was 44.68 kcal/mol and that CDOCKER energy was 43.60 kcal/mol. But the amino acids forming the hydrogen bonds with target and lauric acid were not matching with the active site residues. Capric acid recorded the next lest binding energy with CDOCKER and CDOCKER interaction energy values 38.38 and 39.22 kcal/mol respectively. Binding energy reported for tamoxifen, the commercial drug was even lower than that of lauric acid but the deviation between CDOCKER energy and CDOCKER interaction was high and failed to score an effective interaction with the target ER.

Table 12: Docking scores of coconut compounds and commercial drugs against the protein target ER identified for carcinoma

Protein (PDB ID)	Ligands	(-) CDOCKER energy (kcal/mol)	(-) CDOCKER interaction energy (kcal/mol)	H bond amino acid residues	H bond distance (A°)	Binding energy (kcal/mol)
ER (1ERR)	*1. Coconut compounds*					
	Lauric acid	44.68	43.60	LYS529 LYS529	1.09 2.09	-115.7
	Capric acid	38.38	39.22	HIS524* HIS524*	2.40 2.05	-105.6
	Gallic acid	31.00	29.18	GLU353*	2.30	-81.20
	Caprylic acid	34.37	32.90	HIS524*	2.30	-80.2
	Caffeic acid	26.61	36.84	HIS524*	2.29	-54.8
	2. Commercial drug					
	Tamoxifen	15.76	43.6	ASP351	2.0	-145.13

*Lead amino acid residues in the active sites

4.1.3.2.7 Interaction of coconut phyto compounds with B-cell lymphoma-extra-large transmembrane protein target (Bcl-xl) identified for carcinoma

CDOCKER energy, CDOCKER interaction energy, binding energy and hydrogen bonds formed when the phyto compounds of coconut and commercial drugs were docked with the Bcl-xl target of cancer are summarized in the Table 13.

Lauric acid scored the least binding energy and the value was -93.56 kcal/ mol. Moreover the CDOCKER energy and interaction energy of lauric acid were optimum and the values obtained were 39.25 and 38.46 kcal/mol respectively. ASP133 and ARG139 were identified as the lead amino acid residues in the docking analysis between the phyto compounds of coconut and the target protein. Caffeic acid scored the highest binding energy with a value -46.70 kcal/mol.

Since no specific commercial drug was approved by FDA against the target Bcl-xl comparative study was not performed. Moreover the dock scores obtained in the analysis were not so satisfactory to prove that the phyto compounds selected were effective in inhibiting the Bcl-xl protein.

4.1.4 Active sites prediction and molecular docking of protein targets identified for alzheimer's

4.1.4.1 Selection of active sites

The active site consists of residues that form temporary bonds with the substrate (binding site) and residues that catalyze a reaction of that substrate (catalytic site). A protein usually contains more than one active site. So it is important to find the active site in which the ligands possess an effective interaction. Table 14 depicts the no of active sites identified for alzheimer's target and the one used in the docking analysis. The target APP protein recovered eight active sites while BACE 1&2 recovered four active sites. The possible amino acids involved in the hydrogen bond

Table 13: Dock scores of coconut compounds and commercial drugs against the target Bcl-xl identified for carcinoma

Protein (PDB ID)	Ligands	(-) CDOCKER energy (kcal/mol)	(-) CDOCKER interaction energy (kcal/mol)	H bond amino acid residues	H bond distance (A°)	Binding energy (kcal/mol)
Bcl-xl (3ZK6)	*1. Coconut compounds*					
	Lauric acid	39.25	38.46	ARG139*	2.44	-93.56
	Gallic acid	33.19	31.79	SER106	1.82	-72.49
	Capric acid	32.0	30.91	ARG139* ARG139*	2.37 2.37	-69.97
	Caprylic acid	26.22	25.09	ARG139*	2.22	-69.83
	Caffeic acid	20.63	31.28	ASP133*	2.21	-46.70

*Lead amino acid residues in the active site residues

formation which were obtained from PDB site recorder are also given. More than five amino acids were identified in the active site of protein Beta secretase whereas only two were identified in case of APP. The number of amino acids varied according to proteins and the strength of interactions.

4.1.4.2 Molecular docking

Molecular docking was performed for the targets identified for alzheimer's with the ligands selected. The ligands included the five phyto compounds in coconut and the three commercially available drugs approved by FDA for the medication of alzhimers. The details of commercial drugs used in the study are provided in Table 15.

4.1.4.2.1 Interaction of coconut phyto compounds with the target Amyloid precursor protein (APP) identified for alzheimer's

The best inhibitory capacity of phyto compounds of coconut on APP protein was identified based on the CDOCKER energy, CDOCKER interaction energy, binding energy and hydrogen bond interaction. The results obtained from this docking analysis are summarized in Table 16.

The least binding energy was reported for lauric acid and the value was -101.89 kcal/ mol. Whereas the CDOCKER energy and CDOCKER interaction energy of lauric acid were 29.81 and 28.7 kcal/ mol respectively. Also it was interacting with the amino acid ARG in the 180th position of target APP through three hydrogen bonds. Next to lauric acid, the compound screened with least binding energy was capric acid (109.42 kcal/ mol). Commercial drugs Aricept, Rivastigmine and Razadyne were docked with the target APP since no target specific drugs are available in the market for this particular protein. Among the three drugs, Aricept gave the lowest binding energy but the difference in CDOCKER energy and CDOCKER interaction energies of this drug was not optimum.

	Rivastigmine	DB00989	BACE 1 and BACE 2	Sun pharmaceutical industries ltd	Diarrhea, dizziness, headache, indigestion, loss of appetite

Table 14: Detailes of active site and PDB ID of protein targets involved in alzhemier's

Sl no.	Protein	PDB ID	No of active sites	Active site selected	Amino acids residues in active site
1	Amyloid precursor protein	3KTM	8	Active site 1	Pro173, Gly175
2	Beta secretase 1	1W51	4	Active site 4	Gly34, Asp32, Asp228, Gly230, Gln73, Thr232
3	Beta secretase 2	2EWY	4	Active site 1	Gly50,Asp241,Asp48,Phe124,Tyr87, Gly243,Thr88,Arg248

Table 15 : Details of the FDA approved drugs in use for alzheimer's

Sl. no.	Drugs	Drug bank accession no.	Drug specific protein target	Manufacturing company	Side effects of drugs
1	Aricept (Donepezil)	DB00843	BACE 1 and BACE 2	Pfizer	Nausea, vomiting, diarrhea, loss of appetite, muscle pain, sleep problems (insomnia)
2	Razadyne (Galantamine)	DB00674	BACE 1 and BACE 2	Barr, Impax laboratories etc.	Diarrhea, dizziness, headache, indigestion, loss of appetite
3	Rivastigmine	DB00989	BACE 1 and BACE 2	Sun pharmaceutical industries ltd	Diarrhea, dizziness, headache, indigestion, loss of appetite

Table 16: Dock scores of coconut compounds and commercial drugs against the target APP identified for alzheimer's

Protein (PDB ID)	Ligands	(-) CDOCKER energy (kcal/mol)	(-) CDOCKER interaction energy (kcal/mol)	H bond amino acid residues	H bond distance (A°)	Binding energy (kcal/mol)
APP (3KTM)	*1. Coconut compounds*					
	Lauric acid	29.81	28.75	ARG180* ARG180* ARG180*	2.26 2.43 2.26	-110.89
	Capric acid	27.73	25.68	ARG180*	2.00	-109.42
	Caprylic acid	23.17	21.59	ARG180*	2.15	-101.17
	Caffeic acid	14.22	24.5	ARG180*	1.98	-83.60
	Gallic acid	23.20	20.45	THR83	1.87	-31.97
	2. Commercial drugs					
	Aricept	14.25	29.65	LYS348	2.3	-81.32
	Rivastigmine	11.50	17.80	ASP349	1.9	-42.21
	Razadyne	12.8	20.8	THR83	2.31	-34.8

*Lead amino acid residues in the active site

4.1.4.2.2 Interaction of coconut phyto compounds with protein target Beta secretase (BACE 1 and 2) identified for alzheimer's

Protein targets of alzheimer's like BACE 1 and BACE2 were docked with the coconut phyto compounds and some commercial drugs available in the market.The CDOCKER energy, CDOCKER intraction energy, binding energy and the details about the hydrogen bond interactions from the above mentioned docking analysis are given in Tables 17 and 18.

Least binding energy for protein BACE 1 was identified for lauric acid with a value of 199.27 kcal/mol. Moreover lauric acid scored good CDOCKER energy (45.84 kcal/mol), CDOCKER interaction energy (45.72 kcal/mol) and also formed three hydrogen bonds with lead active site residues (one THR and two ARG (Plate. 5)). For BACE 1 next to lauric, capric acid showed the least binding energy (-191.9 kcal/mol). The other scores of capric acid were also found optimum.

Similarly in case of BACE 2, lauric acid was screened out with the least binding energy (-204.81 kcal/mol). Phyto compounds other than lauric acid such as capric, caprylic, caffeic and gallic acid were also scored optimum binding energies. CDOCKER energy and CDOCKER interaction energy of lauric acid were 46.77 and 44.81 kcal/mol respectively.

Among the three drugs, Rivastigmine showed better scores compared to Aricept and Razadyne (Table 17 and 18). Binding energy of Rivastigmine was optimum compared to others. CDOCKER energy, CDOCKER interaction energy and binding energy of Aricept and Razadyne were not good. The results were indicating that these drugs did not have an effective interaction with the disease target.

Table 17: Dock scores of coconut compounds and commercial drugs against the protein target BACE 1 identified for alzheimer's

Protein (PDB ID)	Ligands	(-) CDOCKER energy (kcal/mol)	(-) CDOCKER interaction energy (kcal/mol)	H bond amino acid residues	H bond distance (A°)	Binding energy (kcal/mol)
BACE1 (1W51)	*1. Coconut compounds*					
	Lauric acid	45.84	45.72	THR232* ARG235* ARG235*	2.19 1.94 1.90	-199.27
	Capric acid	41.21	40.73	THR232* ARG235* ARG2358	2.05 2.07 2.00	-191.97
	Caprylic acid	34.87	33.80	THR232* ARG235* ARG235*	2.05 2.17 2.01	-180.52
	Caffeic acid	25.82	37.18	ARG235* ARG235*	2.03 2.04	-151.54
	Gallic acid	29.65	28.07	THR232* GLN73*	2.07 2.18	-142.32
	2. Commercial drugs					
	Aricept	9.43	52.05	THR72	2.26	-71.57
	Rivastigmine	33.56	40.55	GLY230*	1.91	-59.33
	Razadyne	14.19	32.24	GLN73*	2.32	-2.14

*Lead amino acid residues in the active sites

Table 18: Dock scores of coconut compounds and commercial drugs against the protein target BACE 2 identified for alzheimer's

Protein (PDB ID)	Ligands	(-) CDOCKER energy (kcal/mol)	(-) CDOCKER interaction energy (kcal/mol)	H bond amino acid residues	H bond distance (A°)	Binding energy (kcal/mol)
BACE 2 (2EWY)	*1. Coconut compounds*					
	Lauric acid	46.77	44.81	LYS237 THR88*	2.18 2.02	-204.81
	Capric acid	40.86	39.45	THR88*	2.24	-198.80
	Caprylic acid	36.99	36.70	THR88* ASP241*	2.03 2.06	-191.4
	Gallic acid	34.03	31.41	THR88* THR88* ASP241*	2.12 2.06 2.13	-175.6
	Caffeic acid	24.01	34.46	ARG248*	2.12	-160.1
	2. Commercial drugs					
	Razadyne	7.79	38.56	THR244	2.38	-82.04
	Rivastigmine	28.3	37.69	GLN89	1.99	-24.06
	Aricept	2.57	47.84	THR88*	2.37	-10.76

***Lead amino acid residues in the active sites**

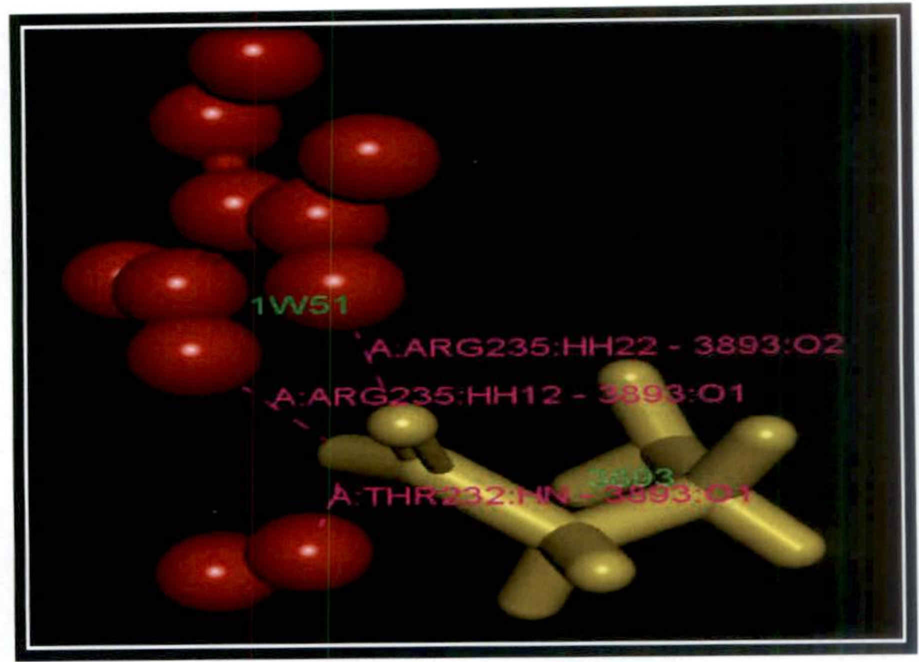


Plate 5: Hydrogen bond interaction between lauric acid and BACE 1

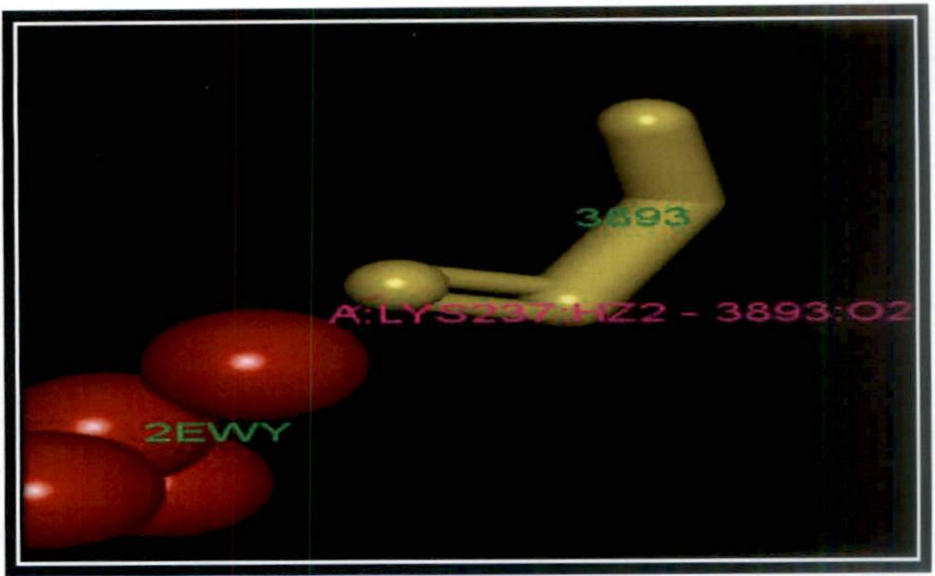


Plate 6: Hydrogen bond interaction between lauric acid and BACE 2

4.1.5 Active site prediction and molecular docking of protein targets identified for diabetes

4.1.5.1 Selection of active sites

Active site of target proteins were selected from the Discovery studio and the important one was identified based PDB site recorder. The active site consists of residues that form temporary bonds with the substrate (binding site) and residues that catalyze a reaction of that substrate (catalytic site). A protein usually contains more than one active site. So it is important to find the active site in which the ligands possess an effective interaction. Table 19 depicts the no of active sites identified for each target of diabetes and the one used in the docking analysis. The number of active sites ranged between two to eight for the different targets. The possible amino acids involved in the hydrogen bond formation which were obtained from PDB site recorder are also given. The target Aldose reductase (ALDR) recovered two active sites while Dipeptidyl peptidase (DPP-IV) recovered eight active sights.

4.1.5.2 Molecular docking

Molecular docking was performed for the targets identified for diabetes with the ligands selected. The ligands included the five phyto compounds in coconut and the two commercially available drugs approved by FDA for the medication of diabetes. The details of commercial drugs used in the study are provided in Table 20. Piogkitazone and Metformin were the commercial drugs selected and both of them had interaction with both the targets ALDR and DPP-IV.

Table 19 : Detailes of the active site and PDB ID of protein targets involved in diabetes

Diseases	Protein	PDB ID	No of active sites	Active sites	Amino acid residues in selected active site
Diabetics	Aldose reductase	1IEI	2	Active site 1	Ser210, Ser214, Lys262, Tyr48, Leu212, Asn 272, Tyr209, Gln183, Arg268
	Dipeptidyl peptidase IV	3F8S	8	Active site 4	Phe 357, Asn 710, Glu 206

Table 20 : Details of the FDA approved drugs in use for diabetes

Sl. No.	Drugs	Protein targets	Drug bank accession no.	Manufacturing company	Side effects of drugs
1	Pioglitazone	Aldose reductase, Dipeptidyl peptidase	DB01132	Advanced Pharmaceutical Services, AQ Pharmaceuticals etc.	Chest pain, decreased urine output, dilated neck veins, extreme fatigue
2	Metformin (Riomet)	Aldose reductase, Dipeptidyl peptidase	DB00331	Ranbaxy pharmaceuticals, Andrx labs etc.	Abdominal or stomach discomfort, cough or hoarseness , decreased appetite, diarrhea

Table 21: Dock scores of coconut compounds and commercial drugs against the protein target ALDR identified for diabetes

Protein (PDB ID)	Ligands	(-) CDOCKER energy (kcal/mol)	(-) CDOCKER interaction energy (kcal/mol)	H bond amino acid residues	H bond distance (A°)	Binding energy (kcal/mol)
Aldose reductase (1IEI)	*1. Coconut compounds*					
	Lauric acid	52.90	52.6	ASP212* SER214* LYS262*	2.15 1.98 1.6	-203.45
	Caprylic acid	47.99	48.71	LYS21* SER210* SER214* SER214*	1.95 2.08 2.09 2.16	-199.98
	Capric acid	49.83	52.63	LYS21* SER214* SER214*	1.96 2.11 1.98	-184.08
	Gallic acid	49.53	48.49	TYR48* SER210* SER214* ASP43 THR19	1.92 2.03 2.39 2.03 2.14	-170.21
	Caffeic acid	37.65	48.26	TYR48* LYS77* SER214* LYS262*	2.27 2.37 2.01 2.46	-169.62
	2. Commercial drugs					
	Pioglitazone	43.39	52.96	SER263	2.86	-42.19
	Metformin	7.482	19.81	GLN18	2.01	-49.86

*Lead amino acid residues in the active sites

4.1.5.2.1 Interaction of coconut phyto compounds with protein target Aldose reductase (ALDR) identified for diabetes

The inhibitory capacity of coconut phyto compounds against aldose reductase target were acessed based on the CDOCKER energy, CDOCKER interaction energy, binding energy and hydrogen bond interactions. Table 21 gives detailes about the different dock scores and hyrogen bonds formed during the docking studies.

Among the five compounds lauric acid scored the least binding energy and the value was -203.45 kcal/mol. CDOCKER energy (52.90 kcal/mol) and CDOCKER interaction energy (52.6 kcal/mol)) of lauric acid were also optimum to indicate that the compound is having an effective interaction with the disease target. More over, lauric acid was forming hydrogen bond with two ative site amino acids, ASP212 and SER214 (Plate. 7). Next to lauric, caprylic, capric, caffeic and gallic acid also scored optimum binding energy, CDOCKER energy and CDOCKER interaction energy. The lead amino acid residues identified for the above interaction were TYR, LYS, ASP and SER.

From the table 21 it is clear that Pioglitazone had comparatively good CDOCKER energy (43.39 kcal/mol) and CDOCKER interaction (52.92 kcal/mol) energy but its binding energy (-42.19 kcal/mol) was high. Similarly Metformin scored poor CDOCKER energy (7.4 kcal/mol) and CDOCKER interaction energy (19.8 kcal/mol). Both the drugs did not show any hydrogen bonding with the lead amino acid residues.

Table 21: Dock scores of coconut compounds and commercial drugs against the protein target ALDR identified for diabetes

Protein (PDB ID)	Ligands	(-) CDOCKER energy (kcal/mol)	(-) CDOCKER interaction energy (kcal/mol)	H bond amino acid residues	H bond distance (A°)	Binding energy (kcal/mol)
Aldose reductase (1IEI)	*1. Coconut compounds*					
	Lauric acid	52.90	52.6	ASP212* SER214* LYS262*	2.15 1.98 1.6	-203.45
	Caprylic acid	47.99	48.71	LYS21* SER210* SER214* SER214*	1.95 2.08 2.09 2.16	-199.98
	Capric acid	49.83	52.63	LYS21* SER214* SER214*	1.96 2.11 1.98	-184.08
	Gallic acid	49.53	48.49	TYR48* SER210* SER214* ASP43 THR19	1.92 2.03 2.39 2.03 2.14	-170.21
	Caffeic acid	37.65	48.26	TYR48* LYS77* SER214* LYS262*	2.27 2.37 2.01 2.46	-169.62
	2. Commercial drugs					
	Pioglitazone	43.39	52.96	SER263	2.86	-42.19
	Metformin	7.482	19.81	GLN18	2.01	-49.86

*Lead amino acid residues in the active sites

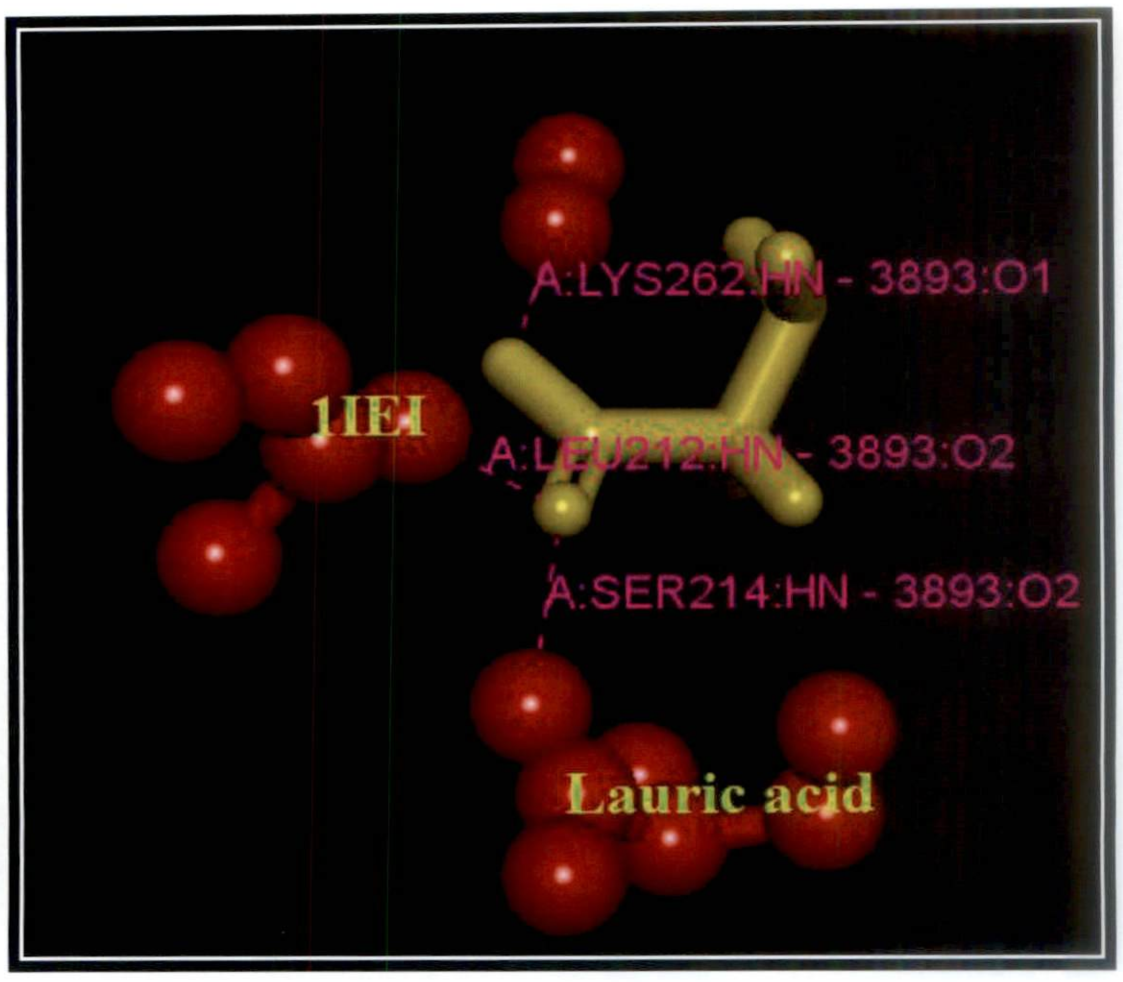


Plate 7: Hydrogen bond interaction between lauric acid and Aldose reductase

4.1.4.2.2 Interaction of coconut phyto compounds with protein target Dipeptidyl peptidase (DPP-IV) identified for diabetes

The interaction between the phyto compounds in coconut with the target protein Dipeptidyl peptidase identified for diabetics in comparison with the commercial drug is provided in Table 29. The binding energy of phyto compounds varies from -7.52 kcal/mol to -52.45 kcal/mol. The different interactive scores recorded for the phyto compounds could not be considered good. Among them, lowest binding energy (-52.45 kcal/mol) was recorded for caffeic acid but its CDOCKER energy (15.55 kcal/mol) and CDOCKER interaction energy (26.79 kcal/mol) were not optimum. The next compound recorded with low binding energy was lauric acid (-45.78 kcal/mol) and its CDOCKER energy and CDOCKER interaction energy were 29.26 and 29.03 kcal/mol respectively. Gallic acid reported the highest binding energy and the value was not at all acceptable (-7.5 kcal/mol). ARG358 and TRP215 were identified as the lead amino acids. Three hydrogen bonds were formed by lauric acid with the ARG amino acid in the 358th position.

Compared to phyto compounds, drug Pioglitazone scored good CDOCKER energy (31.43 kcal/mol) and CDOCKER interaction energy (34.9 kcal/mol). The value of binding energy (-87.85 kcal/mol) was also optimum for Pioglitazone. The drug Metformin indicated a binding energy of -33.62 kcal/mol and the coconut phyto compounds caffeic and lauric acid recorded better binding energies and hydrogen bond interactions than Metformin.

Table 22: Dock scores of coconut compounds and commercial drugs against the protein target DPP-IV identified for diabetes

Protein (PDB ID)	Ligands	(-) CDOCKER energy (kcal/mol)	(-) CDOCKER interaction energy (kcal/mol)	H bond amino acid residues	H bond distance (A°)	Binding energy (kcal/mol)
Dipeptidyl peptidase (3F8S)	*1. Coconut compounds*					
	Caffeic acid	15.55	26.79	ARG358* ARG358*	2.14 2.46	-52.45
	Lauric acid	29.26	29.03	ARG358* ARG358* ARG358*	1.98 2.42 2.21	-45.78
	Capric acid	29.44	28.53	ARG358* ARG358* ARG358*	2.49 2.20 1.94	-25.08
	Caprylic acid	23.36	22.96	ARG358* ARG358*	2.10 1.89	-18.73
	Gallic acid	26.53	24.77	TRP215* VAL303 ARG358*	2.46 2.15 2.11	-7.52
	2. Commercial drugs					
	Pioglitazone	31.43	34.9	TRP215* TRP216	2.12	-87.85
	Metformin	0.28	11.56	LEU1171*	2.13	-33.62

*Lead amino acid residues in the active sites

4.1.6 Active site prediction and molecular docking of protein targets identified for hyperlipidemics

4.1.6.1 Selection of active sites

The active site consists of residues that form temporary bonds with the substrate (binding site) and residues that catalyze a reaction of that substrate (catalytic site). A protein usually contains more than one active site. So it is important to find the active site in which the ligands possess an effective interaction. Table 23 depicts the number of active sites identified for the targets of hyperlipidemics and the one used in the docking analysis. The number of active sites ranged between eight and ten for the different targets. The possible amino acids involved in the hydrogen bond formation which were obtained from PDB site recorder are also given. Three amino acids were identified in the active site of protein HMG Co A reductase whereas five were identified in case of Cholesterol esterase. The number of amino acids varied according to proteins and the strength of interactions.

4.1.6.2 Molecular docking

Molecular docking was performed for the targets identified for hyperlipidemics with the ligands selected. The ligands included the five phyto compounds in coconut and the two commercially available drugs (Aspirin and Atorvastatin) approved by FDA for the medication of hyperlipidemics. The details of commercial drugs used in the study are provided in table 24.

Table 23: Detailes of the active site and PDB ID of protein targets involved in hyperlipidemics

Diseases	Protein	PDB ID	No of active sites	Active sites	Amino acid residues in selected active sites
Hyper lipidemic	HMG Co A reductase	1DQ8	10	Active site 1	Lys722, Ser565, Tyr479
	Cholesterol esterase	1F6W	8	Active site 6	Gly-107,Ala-108, Glu-193, Ser-194, His-435

Table 24 : Details of the FDA approved drugs in use for hyperlipidemics

Sl. no.	Drugs	Drug bank accession no.	Protein (PDB ID)	Manufacturing companies	Side effects of drugs
1	Aspirin	DB00945	HMGCo A reductase, CEase	Bayer healthcare	Ringing in your ears, hallucinations
2	Atorvastatin	DB01076	HMGCo A reductase, CEase	Warner Lambert Company	Cough, difficulty with, swallowing, dizziness, fast heartbeat

4.1.6.2.1 Interaction of coconut phyto compounds with protein target HMG Co A reductase identified for hyperlipidemics

HMGCo A reductase was docked with the different coconut phyto compounds and commercial drugs. Scores obtained for the different parameters to understand the cholestroal inhibiting property of coconut are summarized in Table 25.

Results obtained indicated that the CDOCKER energy, CDOCKER interaction energy and binding energy of all coconut compounds were optimum to have an effective interaction with HMGCo A reductase, the target protein of hyperlipidemics. Among the six ligands, lauric acid possessed the least binding energy (-201.7 kcal/mol) and the values of the CDOCKER energy and CDOCKER interaction energy for lauric acid were 51.55 and 48.72 kcal/mol respectively. The next best score was shown by capric acid and its binding energy (198.5 kcal /mol), CDOCKER energy (46.86 kcal/ mol) and interaction energy (44.67 kcal/ mol) were also optimum. Lauric acid possessed four hydrogen bonds, two with ARG568 and two with LYS722 (Plate. 8) and the bond length formed were 1.9 A^0, 2 $A^{0,}$ 1.2 A^0, and 2.1 A^0 respectively. In case of commercial drugs, both atorvastatin and aspirin scored optimum CDOCKER energy, CDOCKER interaction energy and binding energy. The dock scores of atorvastatin were found almost similar to that of lauric acid.

4.1.6.2.2 Interaction of coconut phyto compounds with protein Cholesterol esterase (CEase) identified for hyperlipidemics

Different dock scores obtained for CEase target when docked with coconut phyto compounds and synthetic drugs are summarized in Table 26. The least binding energy (-107.81 kcal/mol) was recorded for lauric acid whereas its CDOCKER energy and CDOCKER interaction energy were 36.25 and 35.93 kcal/mol respectively.

Table 25: Dock scores of coconut compounds and commercial drugs against the protein target HMGCo A reductase identified for hyperlipidemics

Protein (PDB ID)	Ligands	(-) CDOCKER energy (kcal/mol)	(-) CDOCKER interaction energy (kcal/mol)	H bond amino acid residues	H bond distance (A°)	Binding energy (kcal/mol)
HMGCo A reductase (1DQ8)	*1. Coconut compounds*					
	Lauric acid	51.5	48.72	ARG568* LYS722* LYS722* ARG568*	1.99 2.09 2.1 1.2	-201.7
	Capric acid	46.8	44.67	ARG568* ARG568* LYS722* LYS722*	2.16 2.03 1.90 1.98	-198.5
	Caffeic acid	25.74	37.94	ARG568* ARG568* LYS722*	2.28 2.17 2.05	-195.9
	Caprylic acid	41.2	38.66	ARG568* ARG568* LYS722* LYS722*	1.99 2.08 2.10 2.20	-191.3
	Gallic acid	37.60	35.37	ARG568* LYS722* LYS722*	2.15 2.06 2.39	-188.7
	2. Commercial drugs					
	Atorvastatin	48	51.38	ARG568*	2.04	-193.1
	Aspirin	32.70	38.26	ARG568* LYS722*	2.03	-192.5

*Lead amino acid residues in the active sites

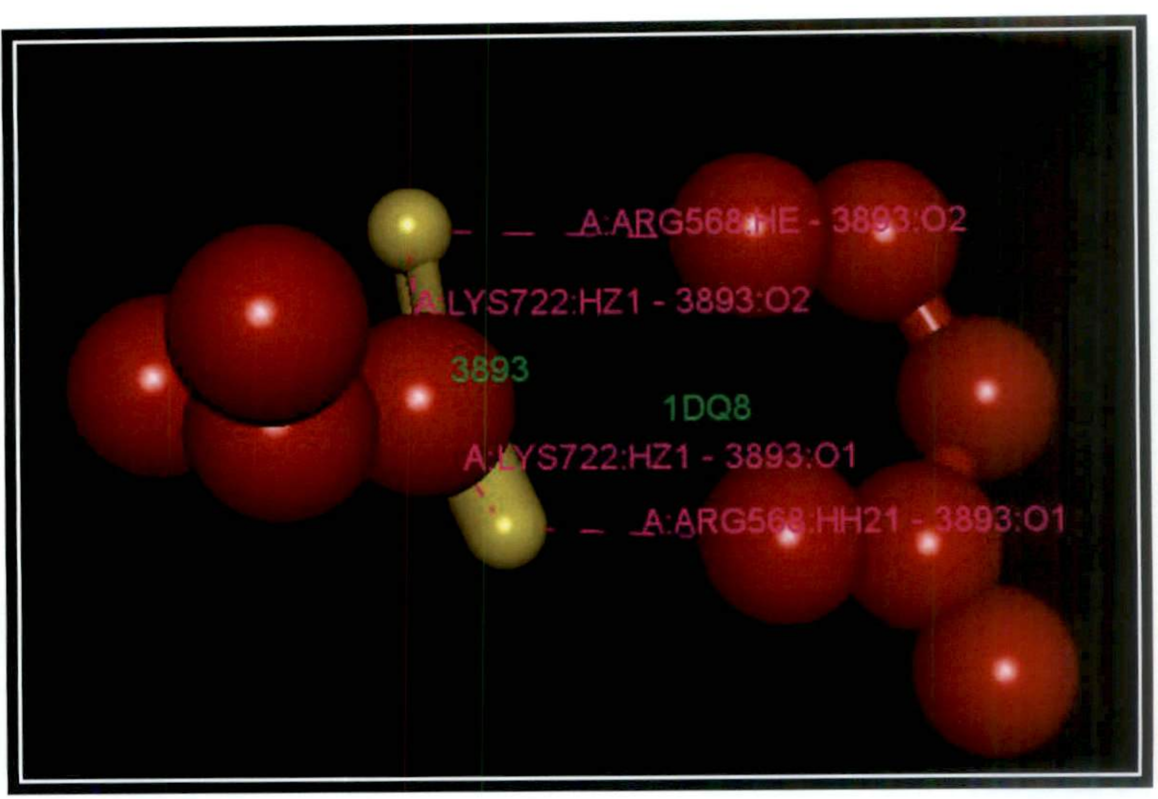


Plate 8: Hydrogen bond interaction between lauric acid and HMG CoA reductase

The lead amino acids identified were GLY107, ALA108, GLU193, TYR125 and SER194. Lauric acid showed only one hydrogen bond interaction while caprylic acid had three. Highest binding energy was reported for gallic acid (34.96 kcal/ mol). Similar to HMG Co A reductase CEase was also docked with the drugs aspirin and atorvastatin for comparative docking analysis (Table 26). No poses was reported for atorvastatin against CEase target. But in case of aspirin the binding energy (-120.94 kcal/mol) was good even compared to phyto compounds. It also possessed good CDOCKER energy and CDOCKER interaction energy.

4.1.7 ADMET evaluation for drug likeness

Considering the CDOCKER energy, CDOCKER interaction energy and binding energy obtained in the docking analysis, the phyto compounds and commercial drugs selected as ligands were forwarded for ADMET analysis. ADMET (Absorption, Distribution, Metabolism, Excretion and Toxicity) properties of compounds are a key determinant to specify whether a molecule can be successfully developed as a drug or not. These properties provide insights in to the compound's pharmacokinetic properties and were thus analysed using Discovery Studio's built in ADMET protocol. The various parameters tested in this study were aqueous solubility, blood brain barrier (BBB) level, hepatotoxicity, absorption level, AlogP and CYPD26.

Details about the pharmacokinetic properties of coconut components and the commercial drugs selected for docking analysis when evaluated through ADMET are given here under.

Table 26: Dock scores of coconut compounds and commercial drugs against the target CEase identified for hyperlipidemics

Protein (PDB ID)	Ligands	(-) CDOCKER energy (kcal/mol)	(-) CDOCKER interaction energy (kcal/mol)	H bond amino acid residues	H bond distance (A°)	Binding energy (kcal/mol)
CEase (1F6W)	*1. Coconut compounds*					
	Lauric acid	36.25	35.93	TYR125*	2.20	-107.81
	Caprylic acid	35.30	35.42	ALA108* ALA108* SER194*	2.09 2.23 2.25	-73.69
	Caffeic acid	19.95	36.45	GLY107* ALA108*	2.16 2.02	-72.28
	Capric acid	31.26	31.56	SER194* SER194*	2.40 2.25	-44.21
	Gallic acid	34.17	31.52	SER194* SER194*	2.12 2.21	-34.96
	2. Commercial drugs					
	Aspirin	27.97	32.74	GLY656	2.04	-120.94
	Atorvastatin	No poses	-	-	-	-

*Lead amino acid residues in the active sites

4.1.7.1 ADMET evaluation of coconut compounds

Table 27 summarizes the results of five coconut compounds used in the docking analysis when evaluated through ADMET descriptors. The optimum levels of each ADMET descriptors are also provided for comparison.

The three MCFAs (lauric, capric and caprylic) and the phenolic compound, caffeic acid responded positively to the pharmacokinetic descriptors. These compounds passed through the ADMET filters by exhibiting scores that falls within the optimum level. But gallic acid, the natural polyphenol in coconut was found toxic in the ADMET analysis. More over the BBB level of gallic acid was 3. It is exceeding the optimum range of Blood Brain Barrier level of 1 to 2 (Table 27). Alog p98 value of gallic acid was also very less. These informations were indicating the fact that even though gallic acid is natural in origin, its pharmacokinetic properties are not favoring the compound to form a potent drug for oral administration.

4.1.7.2 ADMET evaluation of FDA approved drugs for carcinoma

Nine FDA approved carcinoma drugs used in the comparative docking analysis were also filtered through ADMET descriptors. Pharmacokinetic properties of these drugs obtained from ADMET analysis are given in Table 28. The solubility level of Sorafenib and Tamoxifen were very low (1). Only for Letrozole, Gefitinib and Tamoxifen the BBB level was in preferred range. For the other drugs the BBB level was found undefined or too low. More over all the FDA approved drugs for cancer medication were found hepatotoxic. For an effective absorption in the body, the absorption level should be 0. But the drugs like Fluorouracil, Raltitrexed, Methotrexate, Ponatinib and Tamoxifen pharmacokinetic absorption level was ≥ 1.

Table 27: ADME- Toxicity scores of different coconut phyto compounds

Sl. No.	MCFAs (Ligands)	ADMET solubility level (2-4)	BBB level (0-2)	ADMET Alog p98 (upto 5)	Hepatotoxicity (False-non-toxic, True-toxic)	Absorption level (0-Good absorption rate)	CYP2D6 prediction (False-non inhibitor)
1	Caffeic acid	3	2	1.894	False	0	False
2	Capric acid	3	1	3.655	False	0	False
3	Caprylic acid	4	1	2.743	False	0	False
4	Lauric acid	3	1	4.568	False	0	False
5	Gallic acid	4	3	0.733	True	0	False

4.1.7.3 ADMET evaluation of FDA approved drugs for alzheimer's

Aricept, Razadyne and Rivastigmine, the three commonly prescribed FDA approved drugs against dementia were used in the docking analysis. The ADMET results obtained for these compounds are provided in table 28.

Among the three, Aricept and Razadyne were observed to be harmless when analysed through ADMET. The results were positive to all the descriptors. They were non-toxic, have good absorption level and solubility level. Rivastigmine was observed to be hepatotoxic to the body (Table 28) though with other desirable parameters.

4.1.7.4 ADMET evaluation of FDA approved drugs for diabetes

FDA approved commercially available drugs Metformin and Pioglitazone, prescribed for diabetes were analysed through ADMET descriptors and described in Table 28.

Metformin was found hepatotoxic to the body. Also its solubility level was exceeding 4 and BBB value exceeding 2. Meanwhile Pioglitazone gave positive results against the ADMET descriptors. It is non-toxic and the levels of solubility, absorption, BBB etc. were optimum.

4.1.7.5 ADMET evaluation of FDA approved drugs for hyperlipidemics

Aspirin and Atorvastatin were analysed through ADMET descriptors. Both were found hepatotoxic and for Atorvastatin the absorption level exceeded 0 (Table 28). The BBB levels of both drugs were observed to be not in optimum range

Table 28: ADME- Toxicity scores of commercial drugs used for the treatment of selected diseases

Diseases	Drugs (Ligands)	ADMET solubility level (2-4)	BBB level (0-2)	ADMET Alog p98 (upto 5)	Hepatotoxicity (False-non-toxic, True-toxic)	Absorption level (0)	CYP2D6 prediction (False-non inhibitor)
Carcinoma	Letrozole	3	2	2.74	True	0	False
	Gefitinib	2	1	4.08	True	0	False
	Sorafenib	1	4	4.17	True	0	False
	Sunitinib	3	3	1.43	True	0	False
	Fluorouracil	4	3	0.90	True	1	False
	Raltitrexed	3	4	1.71	True	3	False
	Methotrexate	2	4	0.37	True	3	False
	Tamoxifen	1	0	6.31	True	1	False
	Ponatinib	2	3	4.04	True	1	False
Alzheimer's	Aricept	2	1	4.5	False	0	False
	Razadyne	3	2	1.4	False	0	False
	Rivastigmine	3	1	2.5	True	0	False
Diabetes	Metformin	5	3	-0.74	True	0	False
	Pioglitazone	2	1	4.10	False	0	False
Hyperlipidemics	Aspirin	4	3	1.22	True	0	False
	Atorvastatin	2	4	5.55	True	2	False

173727

4.2 VALIDATION THROUGH WET LAB ANALYSIS

Computational tools like molecular docking methods used in the present study highlighted the medicinal values of coconut phyto compounds against different diseases. Among the five phyto compounds (Lauric acid, capric acid, caprylic acid, caffeic acid and gallic acid) selected for the *in silico* analysis, lauric acid gave better results against most of the protein targets selected. To validate these results further wet lab studies were performed.

4.2.1 *In vivo* studies through animal models to validate anti-cholesterol and anti-diabetic properties

In silico results stated that lauric acid was effective in lowering the excessive cholesterol synthesis and diabetic complications by inhibiting the proteins HMG Co A reductase and Aldose reductase. This was further studied using *in vivo* techniques using animal models. The results obtained are described below.

4.2.1.1 Serum lipid profile of male Sprague dawley rats

The serum lipid profile of male Sprague dawley rats fed with coconut oil (2ml/kg of animal), lauric acid low dose (0.5 and 1gm/kg of animal), and the standard drug (Atorvastatin- 10mg/ kg of animal) are summarized in Table 29. Blood cholesterol level in the control group was 80.67 ± 6.56 mg/dl. The level was more or less the same in the animals fed with coconut oil (81.83 ± 5.71 mg/dl).While Lauric acid Low Dose and Lauric acid High Dose administered groups recorded lower levels (70.33 ± 5.43 and 76.50 ± 1.76 mg/ dl) of total cholesterol in blood. The total blood cholesterol level in atorvastatin treated animals was 68.67 ± 4.97 mg/dl.

The level of triglyceride (TG) recorded in control groups (124.8 ± 2.7 mg/ dl) and coconut oil (CO) groups (123.3 ± 1.9 mg/ dl) were more or less the same. While the LLD, LHD and atorvastatin groups had a significantly ($p<0.001$) reduced level of triglyceride. In case of Lauric acid Low Dose and Lauric acid High Dose, the

Table 29: Serum lipid profile (mg/dl) of male Sprague dawley rats fed with coconut oil, lauric acid and atorvastatin, the standard drug

Groups	Total cholesterol (mg/ dl)	Triglycerides (mg/ dl)	HDLc (mg/ dl)	LDLc (mg/ dl)
Control	80.67 ± 6.56	124.8 ± 2.7	22.17 ± 1.72	33.5 ± 5.6
Lauric acid Low Dose (LLD) 0.5gm/kg of animal	70.33 ± 5.43*	113.8 ± 1.7***	24.67 ± 2.25	22.9 ± 5.3*
Lauric acid High Dose (LHD) 1gm/kg of animal	76.50 ± 1.76	114.3 ± 2.3***	28.50 ± 1.38**	25.1 ± 2.5
Coconut Oil (CO) 2ml/kg of animal	81.83 ± 5.71	123.3 ± 1.9	34.33 ± 4.89***	22.8 ± 4.6*
Statin (Std) 10mg/kg of animal	68.67 ± 4.97**	114.0 ± 2.1***	23 ± 3.01	21.2 ± 4.8**

Mean ± SD (n=6). * Significant at $p<0.05$, ** significant at $p< 0.01$ and *** significant at $p< 0.001$

triglyceride levels were 113.8 ± 1.7 mg/ dl and 114.3 ± 2.3 mg/ dl and in atorvastatin administered group, the TG level was 114.0 ± 2.1 mg/ dl.

A significant increase ($p<0.001$) in High Density Lipoprotein (HDL_c) level was found in coconut oil administered group (34.33 ± 4.89 mg/ dl) compared to that of control (22.17 ± 1.72 mg/ dl) (Table 29). HDLc level of Lauric acid High Dose was 28.50 ± 1.38 mg/ dl where as that of Lauric acid Low Dose was 24.67 ± 2.25 mg/ dl. HDLc level did not differ much in statin (23 ± 3.01 mg/ dl) administered group and control.

Low density lipoprotein (LDL_c) level in the control group animals was 33.5 ± 5.6 mg/ dl. A significant ($p<0.05$) reduction was observed in coconut oil (22.8 ± 4.6 mg/ dl) and Lauric acid Low Dose (22.9 ± 5.3 mg/ dl) groups. Whereas atorvastatin fed group recorded highly significant ($p<0.001$) reduction (21.2 ± 4.8 mg/ dl) in LDL_C cholesterol.

4.2.1.2 Liver enzymatic profile of male Sprague dawley rats

Aldose reductase and Sorbitol dehydrogenase enzymes activity were analysed to check the inhibitory activity of coconut phyto compounds against diabetic complications. Meanwhile to validate the hypolipidemic properties of coconut compounds, HMG Co A / Mevalonate ratio and Lipoprotein lipase assay were conducted. Table 30 depicts the carbohydrate and lipid metabolizing enzyme activities in the liver tissues of the different treatment groups.

A significant reduction in the aldose reductase activity was observed in groups fed with coconut oil and Lauric acid High Dose ($p<0.001$). The enzymatic activities observed in these groups of animals were 21.4 ± 2.8 and 26.9 ± 2.0 U/ mg protein (Table 30). The level of aldose reductase enzyme activity in control group was 36.2 ± 3.3 U/ mg protein and that of atorvastatin group was 33.8 ± 2.8 U/ mg protein.

In case of sorbitol dehydrogenase enzyme, not much significant difference was found among the groups of control (37.22 ± 3.14 U/ mg protein), Lauric acid Low Dose (33.05 ± 4.34 U/ mg protein) and atorvastatin (36.90 ± 2.94 U/ mg protein). However the enzymatic activity was significantly low in animals fed with Lauric acid High Dose (30.66 ± 3.70 U/ mg protein) and coconut oil (24.28 ± 2.64 U/ mg protein).

Table 30 also depicts the HMG Co A/Mevalonate ratio of the treated groups. The ratio was found significantly higher in the Lauric acid High Dose fed group (1.14 ± 0.02) compared to control groups (1.00 ± 0.02). The ratio observed for coconut oil and Lauric acid Low Dose fed animals were 1.13 ± 0.02 and 1.12 ± 0.03 respectively. Among lauric acid groups (LHD and LLD) the ratio was found higher with increased dose. In atorvastatin administered group the level was 1.07 ± 0.02 which was not a significant change from that of control group.

With respect to the triglyceride metabolizing enzyme Lipoprotein lipase, a highly significant increase ($p<0.001$) in the activity was observed in coconut oil and Lauric acid High Dose fed groups when compared to control groups. The enzyme activity recorded were 0.80 ± 0.093 and 0.87 ± 0.024 U/ mg protein respectively for CO and LHD. In control groups the enzyme activity was 0.69 ± 0.01 U/ mg protein and the in atorvastatin group was 0.68 ± 0.071 U/ mg protein.

4.2.2 Validation through wet lab analysis using *in vitro* study

Anti-cancerous activity and anti-diabetic activity of lauric acid were validated through cytotoxicity studies on cell lines and gene expression studies through quantitative PCR (qPCR*)*. The two genes used in the expression study were EFGR for cancer and Aldose reductase for diabetes.

Table 30: Liver enzymatic profile (U/ mg Protein) of male Sprague dawley rats fed with coconut oil, lauric acid and atorvastatin, the standard drug

Groups	Aldose reductase (U/ mg protein)	Sorbitol dehydrogenase (U/ mg protein)	HMG Co A/ Mevalonate ratio	Lipoprotein lipase (mU/mg protein)
Control	36.2 ± 3.3	37.22 ± 3.14	1.00 ± 0.02	0.69 ± 0.061
Lauric acid Low Dose (LLD) 0.5gm/kg of animal	30.9 ± 5.3	33.05 ± 4.34	1.12 ± 0.03*	0.77 ± 0.031*
Lauric acid High Dose (LHD) 1gm/kg of animal	26.9 ± 2.0***	30.66 ± 3.70**	1.14 ± 0.02**	0.82± 0.093***
Coconut Oil (CO) 2ml/kg of animal	21.4 ± 2.8***	24.28 ± 2.64***	1.13 ± 0.02*	0.88 ± 0.024***
Statin (Std) 10mg/kg of animal	33.8 ± 2.8	36.90 ± 2.94	1.07 ± 0.02	0.68 ± 0.071

Mean ± SD (n=6). * Significant at $p<0.05$, ** significant at $p< 0.01$ and *** significant at $p< 0.001$

4.2.2.2 Gene expression profile of EGFR and Aldose reductase using qPCR

The expression of EGFR and Aldose reductase genes were evaluated on the cell line HCT 15, after feeding with different levels of lauric acid. The amplification patterns at different Thermal cycles were evaluated to derive the CT values against β actin as the reference gene.

The gene expression was quantified using the ΔΔCt method and accordingly, a positive ΔΔCt indicates down-regulation and a negative ΔΔCt indicates up-regulation (Livak and Schmittgen, 2001). The ΔΔCt values obtained for EGFR and Aldose reductase were recorded when HCT 15 cells were treated with 30 and 50 μg/ml of lauric acid. Results are described in table 31. When the cells were treated with 30 μg/ml of lauric acid, 0.28 fold decrease in the EGFR gene expression was noticed. In the cells treated with 50 μg/ml of lauric acid, expression of EGFR was decreased by 0.46 fold times. In case of Aldose reductase, gene expression was found decreased by 3.75 fold for cells treated with 50 μg/ml of lauric acid and 3.49 fold decrease in case of cells treated with 30 μg/ml of lauric acid. An increase in the concentration of lauric acid was positively affecting the down-regulation of both the genes, Aldose reductase and EGFR.

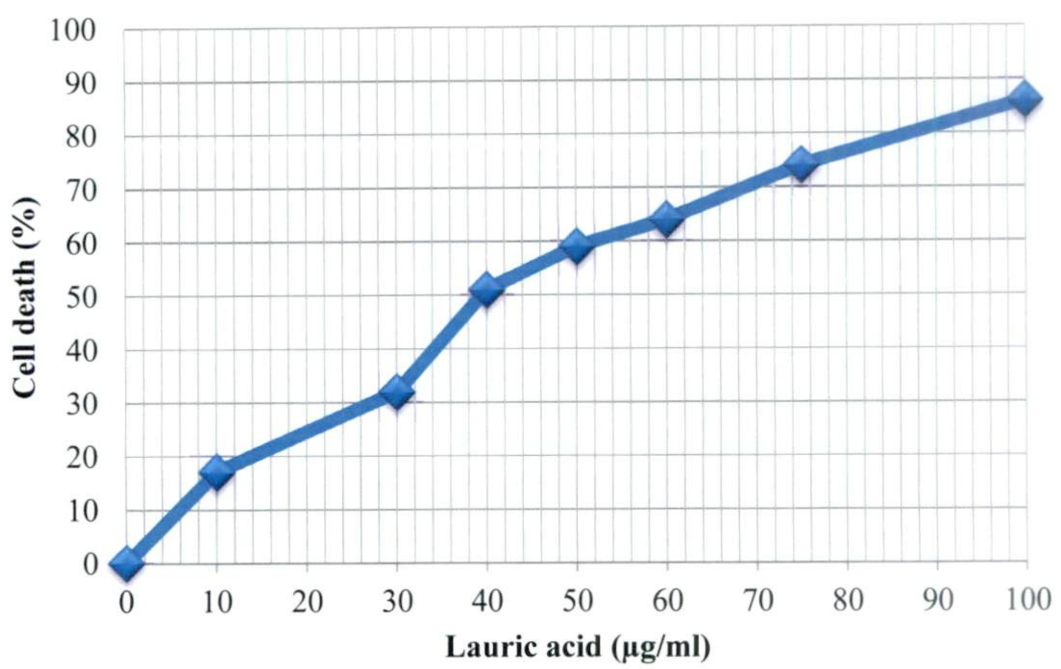


Figure 2: Cytotoxicity induced by lauric acid on HCT 15 cell lines

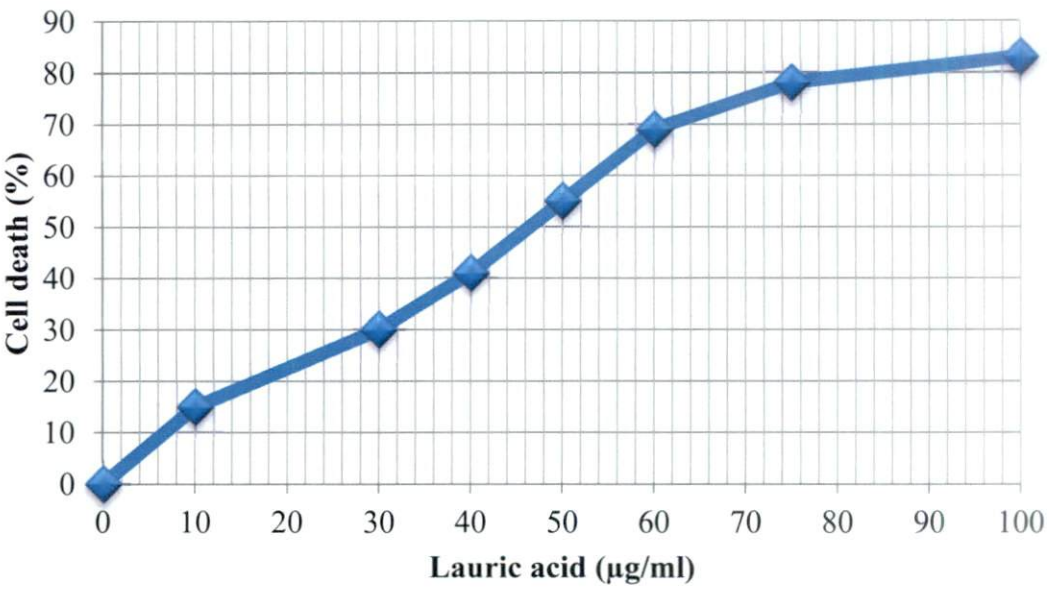


Figure 3: Cytotoxicity induced by lauric acid on HepG2 cell lines

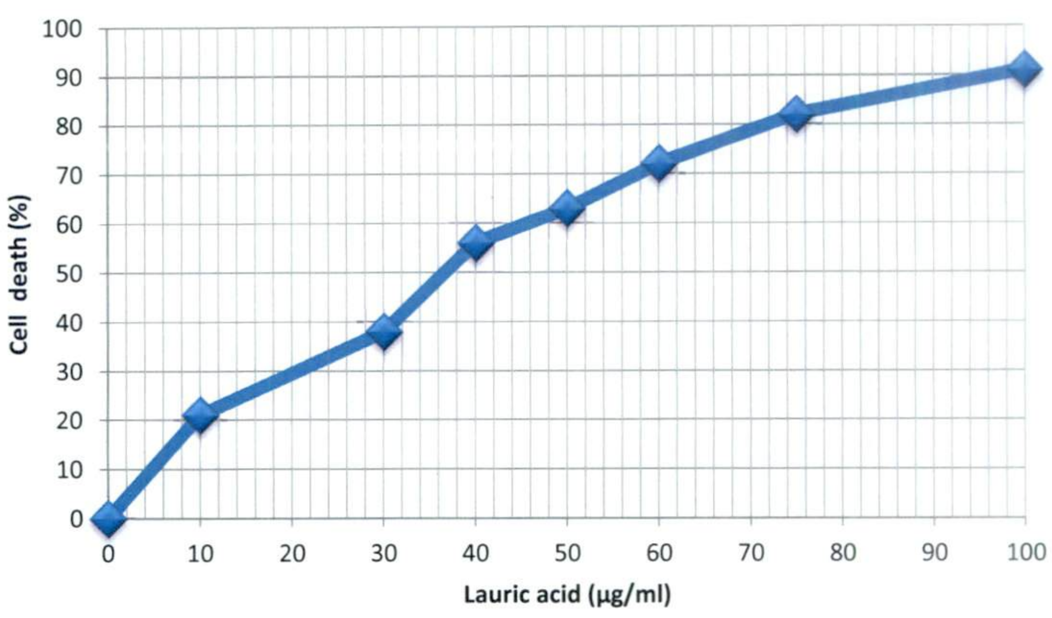


Figure 4: Cytotoxicity induced by lauric acid on RAW 264.7 cell lines

Table 31: Ct and ΔΔCt values obtained for the different genes through qPCR

Different genes	**Treated concentrations**	**Ct values derived from qPCR**		{Ct (Target) - Ct (reference)*} *Ct value for reference= 26.49		**ΔCt (Test) - ΔCt (Control)**
		Control target	**Test target**	**ΔCt** (Test)	**ΔCt** (Control)	**ΔΔCt** (Fold increase (+), Fold decrease (-))
Aldose reductase	Lauric High Dose (50 µg/ml)	28.42	32.17	5.677	1.927	3.75
	Lauric Low Dose (30 µg/ml)	27.59	31.08	4.587	1.097	3.49
EGFR	Lauric High Dose (50 µg/ml)	27.72	28.18	1.687	1.227	0.46
	Lauric Low Dose (30 µg/ml)	27.73	28.02	1.527	1.243	0.284

DISCUSSION

5. DISCUSSION

Coconut is one of the most important oil-yielding crops of the world grown in more than 80 countries of the tropics. It is widely accepted as a multipurpose tree (Enig, 2004). According to Mandal and Mandal (2011), coconut oil is the healthiest oil on earth which has been consumed in tropical countries for thousands of years. For the past three decades, coconut (*Cocos nucifera*) oil has been criticised by the medical community and lay persons alike for contributing to the development of heart disease due to its high saturated fat content. However, after years of study researchers have been unable to link coconut oil consumption with an increased risk of heart problems (Fife, 2005).

Coconut oil is side lined among other vegetable oils mainly due to the health hazards attributed to the saturated fatty acids (92 per cent) present in it. It is often compared with the animal fat due to its high saturated fatty acid content. Literature survey indicates the health benefits of coconut and the exact nature of saturated fatty acids in it. Coconut oil comprises of 72 to 74 per cent saturated Medium Chain Fatty acids (MCFAs) with large number of health benefits. MCFAs in coconut oil include lauric acid, myristic acid, capric acid and caprylic acid. Other than MCFAs some polyphenols such as caffeic acid and gallic acid are also present in coconut having medicinal properties. The exact interaction of these compounds with different disease targets are not much worked out or reported. Thus the present study was taken up as an attempt to unravel the mode of interaction of MCFAs and phenolics in coconut with different disease target proteins so as to highlight the benefits/hazards of such compounds in human health. *In silico* techniques using commercial software, *in vitro* studies using cell lines and *in vivo* experiments with test animals were performed. *In silico* docking helped to evaluate the medicinal values of the phyto compounds through computational tools. Compounds with better performance in *in silico* analysis were further validated through *in vitro* and *in vivo* wet lab experiments.

5.1 *IN SILICO* ANALYSIS

In silico valuation of the selected phyto compounds of coconut were done using the offline tool Discovery Studio 4 of Accelery's USA. Accelrys is a software company with its headquarters in the United States and Discovery Studio is a suite of modeling and simulation programs for life sciences developed by them. Computational techniques can strongly support designing novel, more potent inhibitors by revealing the mechanism of drug-receptor interaction (Tiwari *et al.*, 2012). Molecular docking approaches are routinely used in modern drug designing to identify small molecules by orienting and scoring them in the active binding site of a protein.

Six phyto compounds namely lauric, capric, caprylic, myristic, caffeic and gallic acids were selected as the ligands for the docking analysis. Important proteins involved in the metabolic pathway of diseases like cancer, alzheimer's, diabetics and hyperlipidemics were screened out (Table 4) as targets. Commercial drugs identified for curing the above diseases were also included in the study as ligands for comparison.

The structure of proteins and ligands were down loaded from the data bases like PDB and Pubchem. Later the proteins were prepared through "prepare protein" protocol and ligands were prepared using "prepare ligand" protocol. Prepared ligands were then filtered using Veber and Lipinskis rule of five for their drug likeness properties. For a compound to be considered as a drug molecule, it has to pass the drug likeness property filter as per Veber and Lipinski's rule of five (Lipnski *et al.*, 2001). Except myristic acid, all the other five ligands passed the Lipinski's rule. A log p value of myristic acid was observed as more than 6 (Table 3). Alog p stands for octanol-water partition coefficient and it should not be greater than 5 for compounds to be listed under ideal drugs. Thus further *in silico* evaluation was done

for the remaining five, excluding myristic acid. The compounds were also evaluated for their pharmacokinetic properties through ADMET descriptors.

5.1.1 Molecular docking and significance of dock scores

Molecular docking results were analyzed based on the parameters like CDOCKER score, CDOCKER interaction score, binding energy, amino acids involved in the hydrogen bond interactions and the number of hydrogen bonds formed. During ligand-protein interactions, ligand interacts with the target through different conformations called poses. Among these the most possible conformation facilitating maximum stability to the protein-ligand complex were selected as the best pose (Vijith *et al.*, 2013). The stability of a conformation is determined based on its different dock scores like binding energy, CDOCKER energy, CDOCKER interaction energy and hydrogen bond interactions. The dock scores obtained for the best pose of each ligand with the selected target were recorded to find the interaction.

In a docking experiment, the compound having a dock score with high negative free energy of binding can be considered as a good inhibitor. During interaction, the flexible ligand will go and bind with the proper active site of a rigid protein which is conformationally stable and effective to form a stable complex. This mechanism basically relies on lock and key model where protein is the lock and ligand is the key. The high negative binding energy of a protein-ligand complex indicates that it releases more free energy and moves to a lower, more thermodynamically stable energy state.

Parameters such as CDOCKER energy, CDOCKER interaction energy and hydrogen bond interactions were also evaluated to screen out the best inhibitor. CDOCKER energy is the combined energy produced by the sum of internal ligand strain energy and receptor-ligand interaction energy where, CDOCKER interaction energy is the interaction energy between the protein and ligand and the values of these two parameters indicate the strength of interaction between the proteins and the

ligands. Thus in the present study, besides the least binding energy, compounds with least atomic energy difference between CDOCKER energy and CDOCKER interaction energy were also analyzed.

Hydrogen bonding between a protein and its ligands provide directionality and specificity of interaction that is a fundamental aspect of molecular recognition. The energetics and kinetics of hydrogen bonding need to be optimal in conferring stability to the protein structure and providing the specificity required for selective macromolecular interactions. Amino acids forming hydrogen bonds in molecular docking were compared with those present in the active sites of the protein. Matching amino acids were considered as the active amino acid residues. The number of hydrogen bonds formed with active amino acid residues and the low hydrogen bond length reflects better protein –ligand complex.

5.1.2 Anti-cancerous properties of coconut phyto compounds

In the present study, *in silico* docking analysis helped to analyze the anticancerous properties of MCFAs and poly phenols in coconut oil through computational methods.

Seven proteins such as EGFR, CDK, DHFR, TS, VEGFR, ER and Bcl-xl confirmed to be involved in the metabolic pathway of cancer were selected as the targets for the docking analysis. The five phyto compounds of coconut were docked with each of these proteins in their identified active sites.

The results indicated that all the five phyto compounds of coconut (lauric, capric, caprylic, caffeic and gallic acid) had an effective interaction with the seven targets identified for carcinoma. Among them, the most effective interaction was observed with the target Thymidylate synthase (TS) (Figure 5, 7 and Table 10). TS has become an important target for cancer treatment since it is the most crucial enzyme in the folate pathway and plays significant role in the DNA synthesis and cell

division. The inhibition of TS enzyme will prevent the abnormal synthesis of DNA and it will reduce the severity of different cancers such as colorectal, pancreatic, ovarian, gastric, and breast cancers (Peters *et al.*, 2002). While docking the coconut compounds with TS, their binding energy fell within a range of -193.3 to -213.1 kcal/mol. Lauric acid recorded the lowest binding energy followed by caffeic acid (Figure 5). All the five phyto compounds studied recorded good CDOCKER energy and CDOCKER interaction energy with minimum energy difference in all, except caffeic acid (Figure 7). An energy difference value of 12.72 kcal/ mol was observed for caffeic which is not optimum for an effective interaction. Two hydrogen bond interactions that too with the lead amino acid residue ARG in the 50th position were formed by the phyto compounds with the target TS. This indicates that the chemical bond formation between the ligands and protein were strong and the complex is stable. Among the five phyto compounds, dock scores of lauric acid were found superior to others.

Besides Thymidylate synthases (TS), lauric acid showed positive results in the inhibition of targets like EGFR, DHFR, VEGFR, ER and Bcl-xl involved in carcinoma. It was an effective inhibitor of the above mentioned targets with its optimum scores in all dock parameters including binding energy, CDOCKER energy and CDOCKER interaction energy. It also recorded minimum variability among CDOCKER energy and CDOCKER interaction energy between 0.5 to 3.8 kcal/mol (Figure 7). The variability permitted in better interaction is within 10.

Caffeic acid in coconut oil also recorded good dock values with all the seven targets selected for carcinoma. Very good interactions were observed with four cancer targets such as CDK, TS, VEGFR and EGFR, in which the highest interaction in the latter. However, the difference in CDOCKER energy and CDOCKER interaction energy observed for the binding of caffeic acid with the cancer targets were not satisfactory. Permitted value below 10 was observed only for two targets,

CDK and DHFR. It was also observed that commercial drug Methotrexate for DHFR also recorded an energy difference of near 10, though with a better binding energy (Figure 6 and 8).

The dock results obtained for the target specific drugs were also compared with those of phyto compounds. As a drug, expectations were fulfilled by Methotrexate, a DHFR inhibitor and Raltitrexed, an inhibitor of TS. They recorded good binding energy, CDOCKER and CDOCKER interaction energy with minimum energy difference (Figure 6 and 8). The dock scores of these drugs were even better than those obtained for the natural coconut compounds. The interactions of other drugs indicated high variation among CDOCKER energies.

The anticancerous properties of coconut have been reported in a few research works. Reddy *et al.* (1984) reported that coconut oil had inhibitory action on colon tumors induced by azoxymethane. Fauser *et al.* (2013) reported that lauric acid can induce apoptosis in Caco-2 intestinal cell lines by arresting the cells growth in the S and G2/M phases. An important anti-cancer property of coconut oil is due to the ability of normal cells to utilize medium-chain fats for energy while cancer cells are unable to do so. Thus, by switching one's diet from a sugar and carbohydrates to coconut oil, one can starve cancer cells for energy while providing healthy cells the energy that it needs (Dayrit, 2014). The results obtained in the present *in silico* study supports the fact that the MCFAs of coconut oil mainly lauric acid can act as an inhibitor of protein targets inducing carcinoma.

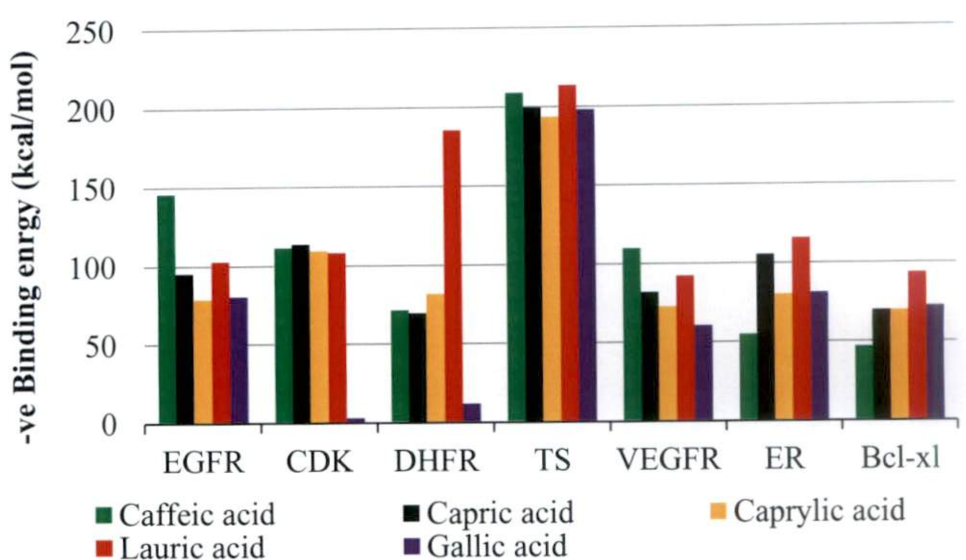


Figure 5: Interaction of (-ve Binding energy) phyto compounds with the different targets of carcinoma

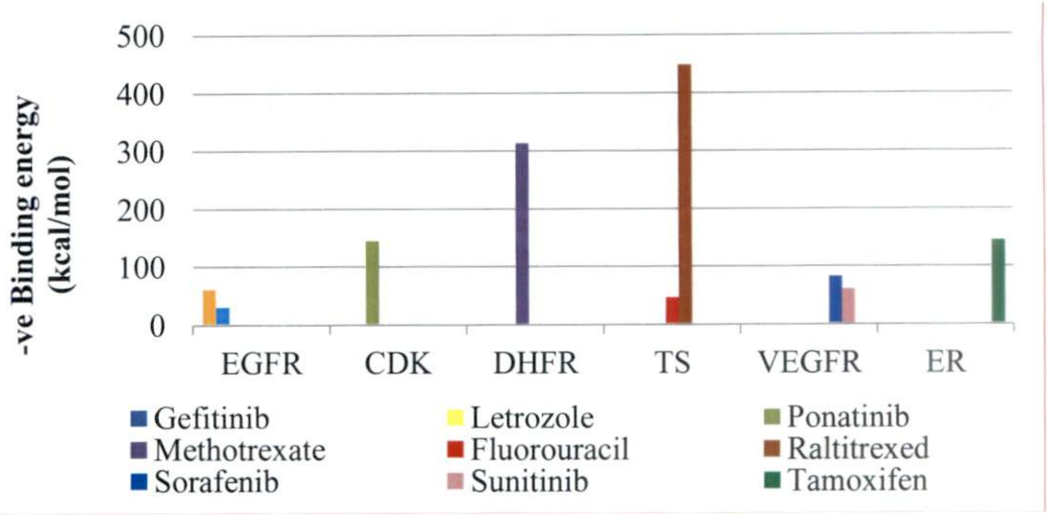


Figure 6: Interaction of (-ve Binding energy) commercial drugs with the different targets of carcinoma

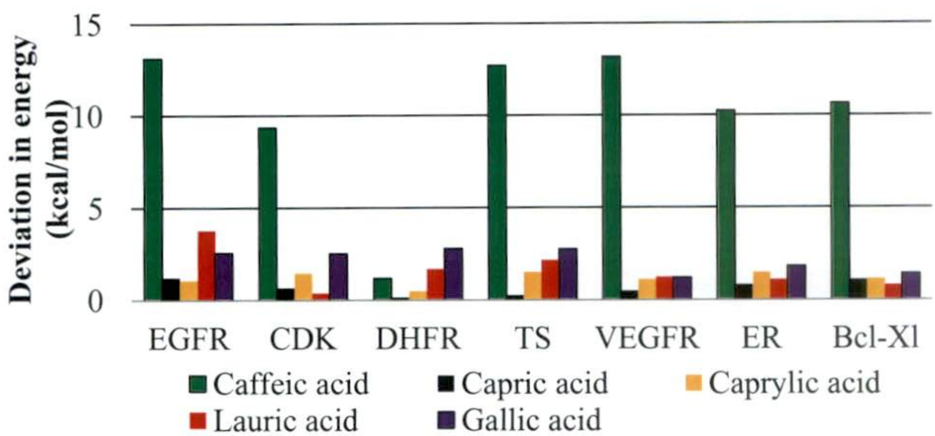


Figure 7: Deviation in CDOCKER and CDOCKER interaction energy observed during interaction of phyto compounds with different targets of carcinoma

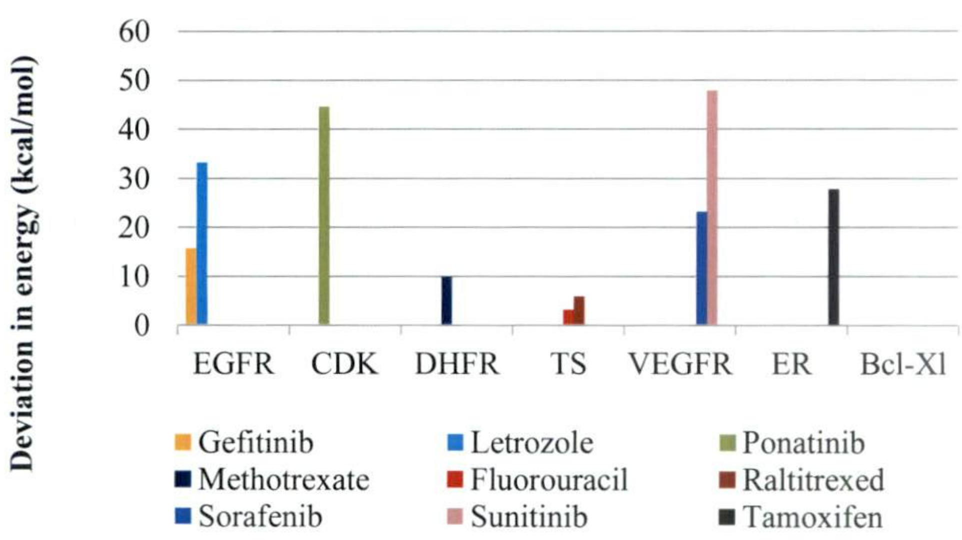


Figure 8: Deviation in CDOCKER and CDOCKER interaction energy observed during interaction of commercial drugs with different targets of carcinoma

5.1.3 Anti-alzheimer's properties of coconut phyto compounds

The *in silico* docking analysis helped to analyze the anti-alzheimer's properties of MCFAs and poly phenols in coconut oil through computational methods.

Three proteins such as APP, BACE 1 and BACE 2, involved in the metabolic pathway of neurogenic disorders like alzheimer's were selected as the targets in the docking analysis. Alzheimer's disease is mostly associated with the formation of beta amyloid (Aβ) plaques and tangles in the brain. Amyloid Precursor Protein (APP) is best known as the precursor molecule whose proteolysis generates beta amyloid (Aβ) whose amyloid fibrillar form is the primary component of amyloid plaques. The enzymes Beta Secretase 1 and 2 (BACE 1 and BACE 2) cleave APP sequentially. Levels of these enzymes have been shown to be elevated in the far more common late-onset of Alzheimer's. Drugs to block this enzyme (BACE inhibitors) would prevent the buildup of beta-amyloid and may help slow or stop Alzheimer's disease (Gupta *et al.,* 2010). Five phyto compounds of coconut were docked with these proteins in their identified active sites to check whether these natural compounds have any inhibitory action in alzheimer's.

The dock results were found positive in case of all the three proteins selected. The coconut phyto compounds were collectively expressing positive trend in the suppression of BACE 1 and BACE 2 proteins of alzheimer's with high binding energy ranging between 180 to 200 kcal/mol and good CDOCKER energy and CDOCKER interaction energy with minimum energy difference (Figure 9, 10 and Table 17, 18).

The dock scores for APP were relatively low as compared to the other two targets. However the binding energy observed was around 100. The deviation observed between CDOCKER energy and CDOCKER interaction was very low (<3)

for all phyto compounds except for caffeic acid. Strong hydrogen bonds were also observed involving active amino acid residues which make the protein-ligand complex more stable (Table 16, 17 and 18). Among the phyto compounds lauric acid was found most effective in all the targets, BACE 1 and BACE 2 and APP and the dock scores recorded for lauric acid were far better than that of nonspecific commercial drugs analysed.

No target specific commercial drugs could be identified from the databank for alzheimer's disease. Hence nonspecific drugs were used in the docking studies for comparison. This may be the reason for the poor interaction reflected in binding energy, CDOCKER energy and CDOCKER interaction energy recorded for the drugs Aricept, Rivastigmine and Razadyne (Figure 9 and 10).

The importance of coconut oil and its effects on neurological disorders were promoted from ancient times. Veech *et al.* (2001) reported that MCFAs in coconut can act as alternative source of energy to brain neurons in alzheimer's patients. Reger *et al.* (2004) claimed that consumption of medium chain fatty acids led to immediate improvement in brain function in patients with milder forms of Alzheimer's. Newport (2008) reported the effects of medium- chain fatty acids, which act like an alternative fuel in the insulin-deficient Alzheimer's brain and that it can sometime, reverse or at least stabilize the disease.

The present study thus emphasize the fact that coconut oil, being natural in origin and pure in its components, could be effective in reducing or preventing the incidence of neurological disorders like alzheimer's through its MCFAs.

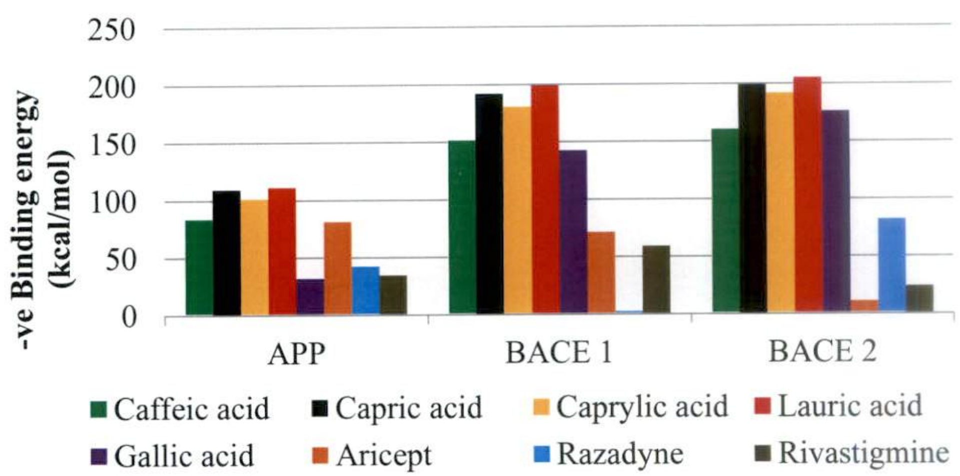


Figure 9: Interaction (-ve Binding energy) of phyto compounds and commercial drugs with different targets of alzheimer's

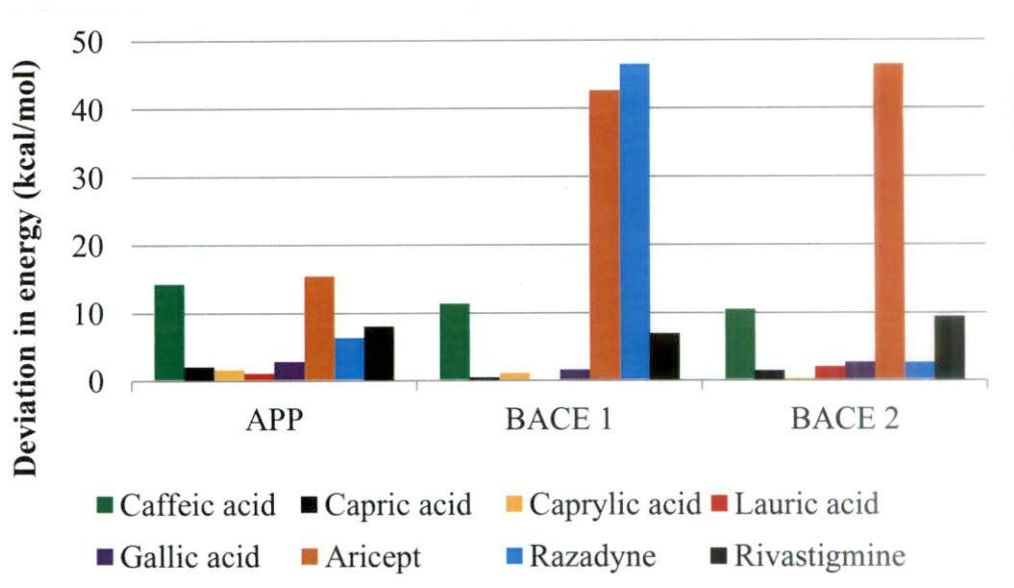


Figure 10: Deviation in CDOCKER and CDOCKER interaction energy observed during interaction of phyto compounds and commercial drugs with different targets of alzheimer's

5.1.4 Anti-diabetic properties of coconut phyto compounds

In silico docking analysis helped to analyze the interaction of MCFAs and poly phenols in coconut oil with the protein targets involved in diabetes.

Two protein targets selected for the analysis were Aldose reductase and Dipeptidyl peptidase (DPP). The selected phyto compounds of coconut were docked with each of these target proteins in their identified active sites.

Diabetes mellitus is associated with defective sugar metabolism and it leads to painful neuropathy, heart disease and kidney failure. Aldose reductase catalyzes the NADPH-dependent conversion of glucose to sorbitol, the first step in polyol pathway of glucose metabolism. Glucose concentrations are often elevated in diabetics and aldose reductase has long been believed to be responsible for diabetic complications. DPP4 is also an important enzyme which plays a major role in glucose metabolism. Inhibition of the DPP-4 enzyme prolongs and enhances the activity of incretins in insulin secretion and blood glucose control regulation (Akhila *et al.*, 2012).

In the *in silico* analysis coconut compounds collectively proved that they were effective in inhibiting the expression of aldose reductase enzyme. All compounds scored good CDOCKER energy and CDOCKER interaction energy with minimum energy difference (Figure 12). Their binding energy varied from -169.62 to -203.45 kcal/ mol (Figure 11). The phyto compounds were forming strong hydrogen bonds with the protein through active site amino acid residues (SER210, SER214, LYS21, TYR48) and the number of hydrogen bonds were more than two for each protein. However, the coconut compounds were not able to form stable ligand-protein complexes with the target protein DPP 4 as reflected in their poor dock scores (Figure 11, 12 and Table 22).

enzymes like Aldose reductase, thus highlighting the role of coconut compounds as a natural inhibitor of diabetic's disease.

The dock results were found superior for lauric acid against Aldose reductase, since the difference in CDOCKER energy and CDOCKER energy were almost nill. More over lauric acid possessed the lowest binding energy and its value was -203.45 kcal/ mol (Table 21 and Figure 11, 12).

FDA approved drugs commonly prescribed for diabetes mellitus are Pioglitazone and Metformin. These drugs were also docked with Aldose reductase and DPP since no target specific drugs were available for these enzymes. The CDOCKER energy and CDOCKER interaction energy of Pioglitazone was good for both the targets. Moreover it was found that the binding energy possessed by this drug was far better than phyto compounds against Dipeptidyl peptidase (Table 22). But the other drug, Metformin recorded low interaction with the proteins Aldose reductase and DPP during *in silico* analysis. The drug metformin might be having better biological activity though with low dock scores. This might have been confirmed through QSAR analysis and pharmacophore modeling during the drug development processes (Kubiny, 1997). Metformin is reported to have its anti-diabetic property through suppressing glucose production by the liver.

Aldose reductase activity increases the blood glucose concentration in diabetic patients, while an inhibitor will block the expression of this enzyme to reduce the disease risk by lowering glucose synthesis (Raj *et al.*, 2014). The present *in silico* docking results highlights the importance of coconut phyto compounds in effectively lowering the diabetic complications by inhibiting the expression of enzymes like Aldose reductase, thus highlighting the role of coconut compounds as a natural inhibitor of diabetic's disease.

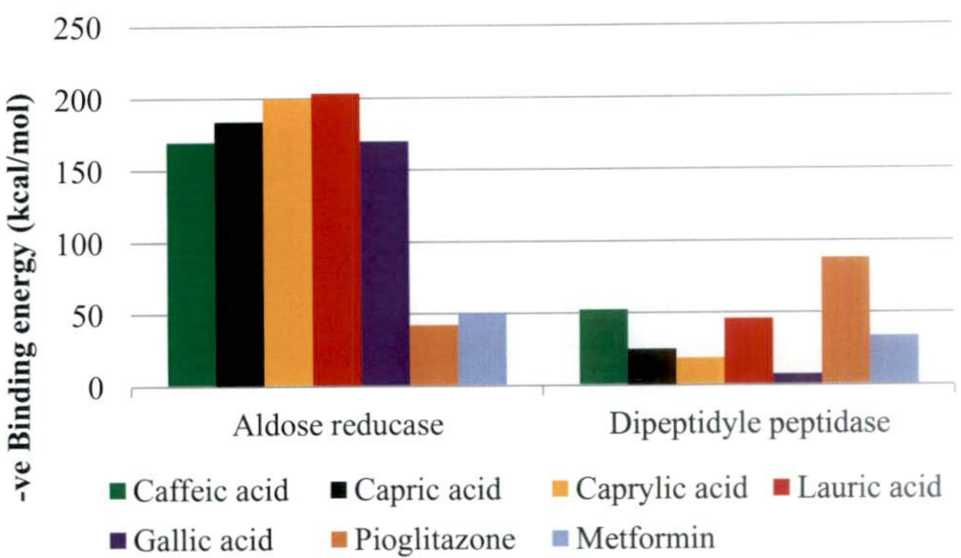


Figure 11: Interaction (-ve Binding energy) of phyto compounds and commercial drugs with different targets of diabetes

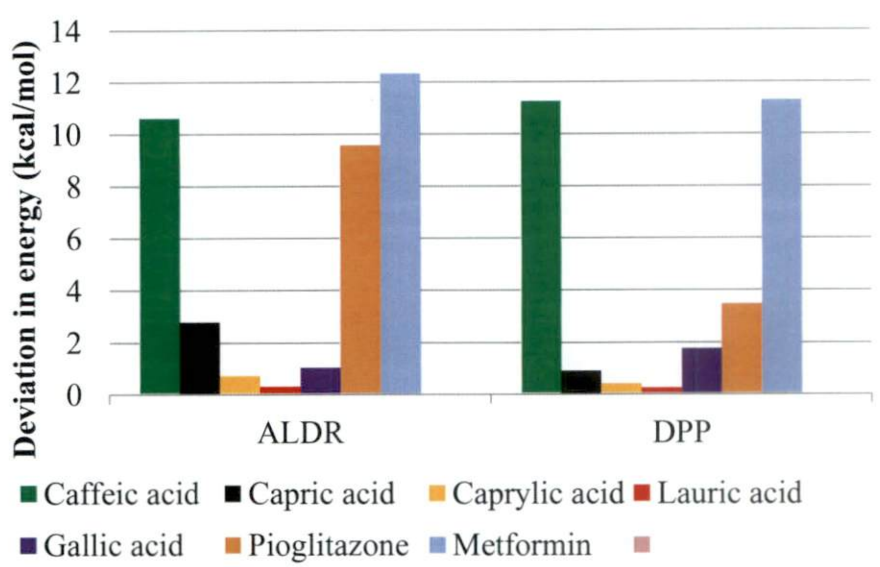


Figure 12: Deviation in CDOCKER and CDOCKER interaction energy observed during interaction of phyto compounds and commercial drugs with different targets of diabetes

5.1.5 Coconut phyto compounds against hyperlipidemics

Two protein targets selected for the analysis are HMGCo A reductase and Cholesterol esterase. All the five coconut compounds were docked with each of these proteins in their identified active sites.

In silico results indicated that natural compounds in coconut plays a significant role in reducing the cholesterol level by inhibiting the protein target HMGCo A reductase. HMGCo A reductase is a rate controlling enzyme in mevalonate pathway that produces cholesterol and other isoprenoids. If this particular enzyme is over expressed, it will lead to the abnormal synthesis of cholesterol and leads to hyperlipidemic complications. The binding energy obtained by the coconut compounds against HMGCo A reductase falls in a range of -188.7 to -201.7 kcal/ mol (Figure 13). They scored good CDOCKER energy and CDOCKER interaction energy with optimum difference in their energy level (Table 25 and Figure 14). More over minimum three hydrogen bond interactions were formed by each protein involving the active site amino acids such as ARG568 and LYS722 (Table 25). By comparing the overall results of HMGCo A reductase, lauric acid was identified as best compound which was interacting most effectively with the target.

Similarly in case of Cholesterol esterase, lauric acid interacted most efficiently with least binding energy and also scored optimum CDOCKER energy and CDOCKER interaction energy (Table 26) with minimum energy difference. The binding energies were not satisfactory for the other phyto compounds.

Aspirin and Atorvastatin are the commercially available FDA approved drugs for hyperlipidemic complications. They were also docked with the target proteins for comparative analysis. Atorvastatin is a target specific drug for HMG Co A reductase coming under statin family. The dock scores obtained for the interaction between statin and the target were almost similar to scores possessed by lauric acid.

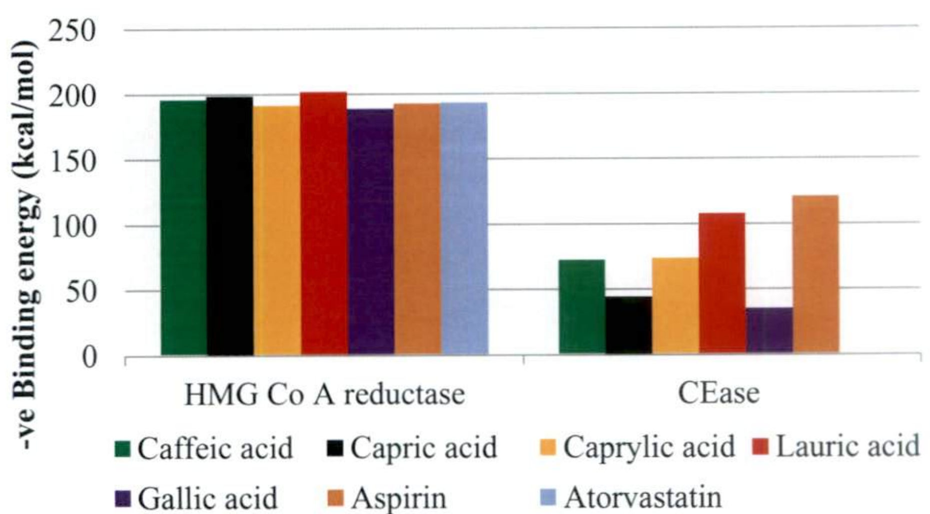


Figure 13: Interaction (-ve Binding energy) of phyto compounds and commercial drugs with different targets of hyperlipidemics

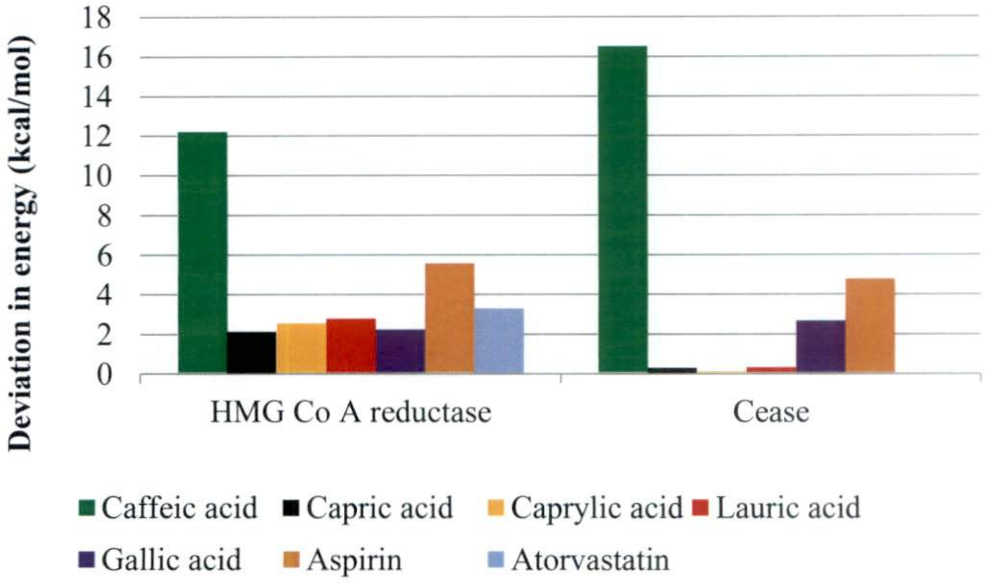


Figure 14: Deviation in CDOCKER and CDOCKER interaction energy observed during interaction of phyto compounds and commercial drugs with different targets of hyperlipidemics

Aspirin also showed good interaction with both the targets. Its binding energy was too good with CEase (Table 26, Figure 13).

HMG-CoA reductase inhibitors, coming under the family of statins are a class of cholesterol lowering drugs that inhibit the enzyme HMG-CoA reductase expression which plays a central role in the synthesis of cholesterol (Lewington *et al.*, 2007). The best-selling statin is atorvastatin, which in 2003 became the best-selling pharmaceutical in history (Sweetman, 2009). As of 2010, a number of statins are on the market such as atorvastatin (Lipitor), fluvastatin (Lescol), lovastatin (Mevacor, Altocor) etc. In the present study, *in silico* dock results of this drug was found good against HMG Co reductase which was almost similar to the scores possessed by the coconut phyto compounds. And these results highlight the property of coconut compounds in lowering or inhibiting cholesterol synthesis.

5.1.6 The drug likeness properties of coconut phyto compounds and commercial drugs

ADMET is an abbreviation in pharmacokinetics and pharmacology for "absorption, distribution, metabolism, and excretion," and describes the disposition of a pharmaceutical compound within an organism. The five criteria all influence the drug levels and kinetics of drug exposure to the tissues and hence influence the performance and pharmacological activity of the compound as a drug.

A new chemical compound to prove it as an actual drug it has to pass through the ADMET descriptors. The ADMET results describe the fate of the compound in human body. Each ADMET parameter has its own range to filter the compounds. The coconut phyto compounds and commercial drugs used in the docking analysis were analysed through ADMET parameters.

Phyto compounds are natural in origin and it was found out that except gallic acid all other compounds passed the filter by obtaining optimum ADMET scores. Their solubility, absorption and BBB levels were within the range (Table 27). BBB level predicts the blood brain barrier penetration of a molecule. BBB is defined as the ratio of the concentration of solution on both sides of the membrane (blood-brain). Also the phyto compounds recorded optimum scores in Alog p and CYP2D6. ADMET CYP2D6 binding is used to predict the P_{450} 2D6 enzyme inhibition and reports whether or not a compound is likely to be an inhibitor. These compounds were also found non-hepatotoxic to the body.

But in case of commercially available drugs, even though they are widely used for the treatment of diseases they did not show satisfactory results in ADMET analysis. Some of the drugs were observed to be hepatotoxic to body (Table 28). Their synthetic origin may be the reason for these unsatisfactory results. However, the drug becomes commercially viable due to its better biological activity and positive results in clinical trials. Owing to its positive effects in disease control, negative side effects are not valued seriously. This might be the reason for advising the patients to take routine liver function test and renal tests while prescribing the drugs like statins and aspirins having hepatotoxicity and high Alog p values.

5.2 WET LAB ANALYSIS FOR VALIDATION

Wet lab studies were performed based on the positive results obtained in the *in silico* analysis. The anti- diabetic and hypolipidemic properties of coconut phyto compounds were validated through animal studies and anti-cancerous properties through cell line cultures. Further gene level expression was analysed through q*RTPCR.*

5.2.1 *In vivo* animal studies to validate hyperlipidemic properties

Hyper cholesterolemia is one of the major risk factor of coronary heart disease. An excessive intake of cholesterol rich food often leads to gradual increase in the serum cholesterol level and cause blood lipid abnormalities. Kaunitz (2001) reported that medium chain fatty acids (MCFAs) are easily digestable, not cerenlate in the blood stream and not stored but are readily oxidized in the body to provide quick energy. Coconut oil comprises of 50 per cent of lauric acid as MCFAs. Up on digestion, lauric acid readily oxidized to monoester form called monolaurin which is a rich source of energy and it elevates the metabolic activity of body. When the body is metabolically active chances for deposition of cholesterol or triglyceride in the blood stream is thought to be reduced. The *in silico* analysis in the present study also highlighted the positive interaction of lauric acid with the targets identified for hyperlipidemics. To validate the *in silico* results animal experiments were conducted by feeding them with coconut oil and lauric acid along with the standard drug, atorvastatin.

In the animal study, administration of Coconut Oil (CO) was found no significant effect in reducing the cholesterol and triglyceride level but it acts as a promoter of High Density Lipoprotein cholesterol (HDLc) and reduces LDLc (Figure 15, Table 29). Similarly lauric acid enhanced HDL_c level and reduced LDLc activity (Figure 15). It also reduced Total cholesterol and Triglycerides level in animals when fed with different doses. The reduction in lipid profile was irrespective of dose in the case of lauric acid. However lauric acid has shown higher potential than CO. As a standard drug prescribed for the cholesterol lowering in hyperlipidemic diseases, 'atorvastatin' reduced the level of cholesterol and triglyceride in the blood and tissues of rats.

HDLc is considered as good cholesterol because they can transport fat molecules like cholesterol and phospholipids out of artery walls and thereby reducing

the risk of hyperlipidemia (Sirtori, 2006). LDLc on the other hand considered "bad" as the level of this cholesterol molecules are well correlated with the cardiovascular events. Kurup and Rajmohan (1994) reported that the level of HDLc was high among animals consumed coconut oil. These studies are in agreement with the present observations.

In parallel with these observations, HMG Co A/Mevalonate ratio of liver enzymatic profile (Table 30) is also found high among the animal groups fed with coconut oil and lauric acid. An elevated HMG CoA /Mevalonate ratio indirectly indicates that the activity of HMG CoA enzyme reductase activity is low. HMG-CoA reductase is the rate-controlling enzyme of cholesterol synthesis pathway. The decrease in the enzymatic activity of HMG Co A reductase slows down the conversion of HMG Co A protein to mevalonate there by inhibiting the synthesize of cholesterol. In the present study, HMG /Mevalonate ratio was good in lauric acid treated groups compared to control and groups treated with atorvastatin. This indirectly indicates the efficacy of lauric acid to inhibit cholesterol synthesis which supports the *in silico* results. However lipid profile results indicated that statins are found more effective than the coconut oil or lauric acid in reducing total cholesterol. This contradiction could be explained by the fact that atorvastatin does not increase HDLc cholesterol while coconut oil and lauric acid significantly increase HDL cholesterol level. Total cholesterol is the sum of LDL, VLDL and HDL cholesterol and HDL cholesterol is formed during the metabolic process. Statin as reported as an inhibitor of HMG CoA reductase might have better effect on total cholesterol level by inhibiting its synthesis but neutral effects in HDLc metabolism. However lauric acid and coconut oil having comparatively same effect as Statin in inhibiting the HMG CoA reductase may be more influential as it increases HDLc formation during metabolism. So total cholestroal in blood found high for coconut oil and lauric acid compared to statin.

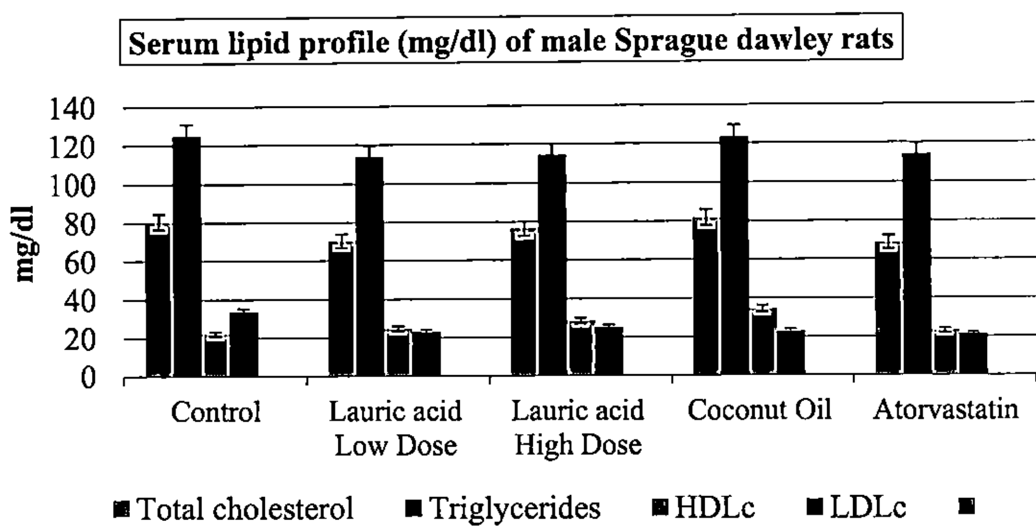


Figure 15: Serum lipid profile (mg/dl) of male Sprague dawley rats showing the hypolipidemic activity of coconut oil, lauric acid and atorvastatin

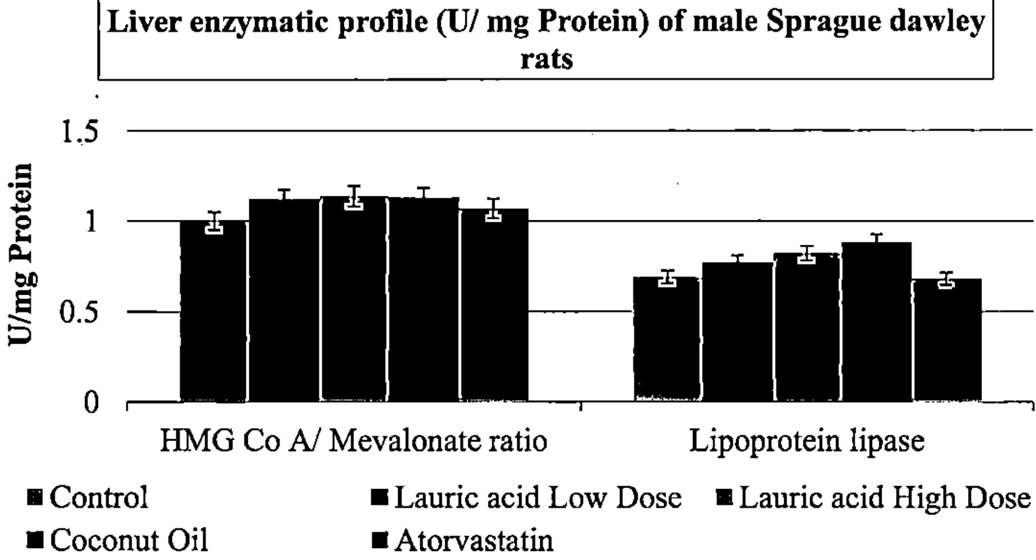


Figure 16: Liver enzymatic profile (U/ mg Protein) of male Sprague dawley rats showing the hypolipidemic activity of coconut oil, lauric acid and atorvastatin

Lipoprotein lipase hydrolyzes triglycerides into free fatty acids and one monoacylglycerol molecule. The activity of this enzyme is recorded high in coconut oil (CO) fed groups which is the same as that of lauric acid low and high dose fed groups (Table 30 and Figure 16). Increased level of lipoprotein lipase indicates that the excess amounts of triglycerides are converted to fatty acids and glycerol. However triglyceride level in coconut oil fed group animals are comparatively higher than that of lauric acid treated group. It has been assumed that enhanced lipoprotein lipase activity shown by coconut may be contributed by its lauric acid content. The other fatty acids present in the CO may be contributory to the formation triglyceride rich lipoproteins. This could be the reason why CO treated groups which possess similar lipoprotein lipase activating efficacy to lauric acid have higher level of triglycerides.

5.2.2 *In vivo* animal studies to validate anti-diabetic properties

Aldose reductase and sorbitol dehydrogenase are enzymes associated with the polyol pathway. Activation of polyol pathway due to increased aldose reductase activity which catalyzes the reduction of glucose to sorbitol is one of the several mechanisms that have been implicated in the development of various secondary complications of diabetes (Reddy *et al.*, 2008). As the concentration of sorbitol increases a gradual elevation in the level of sorbitol dehydrogenase would occur since this enzyme is responsible for the further conversion of sorbitol to fructose in polyol pathway. Except in the diabetic conditions the activity of these two enzymes are normal in the liver.

The results obtained from the animal study supports the *in silico* results by showing a reduced level of aldose reductase and sorbitol dehydrogenase enzymatic activity in groups fed with coconut oil and lauric acid (Figure 17 and Table 30). The diabetic complications of these groups of animals are negligible compared to control group since the activities of both enzymes were inhibited.

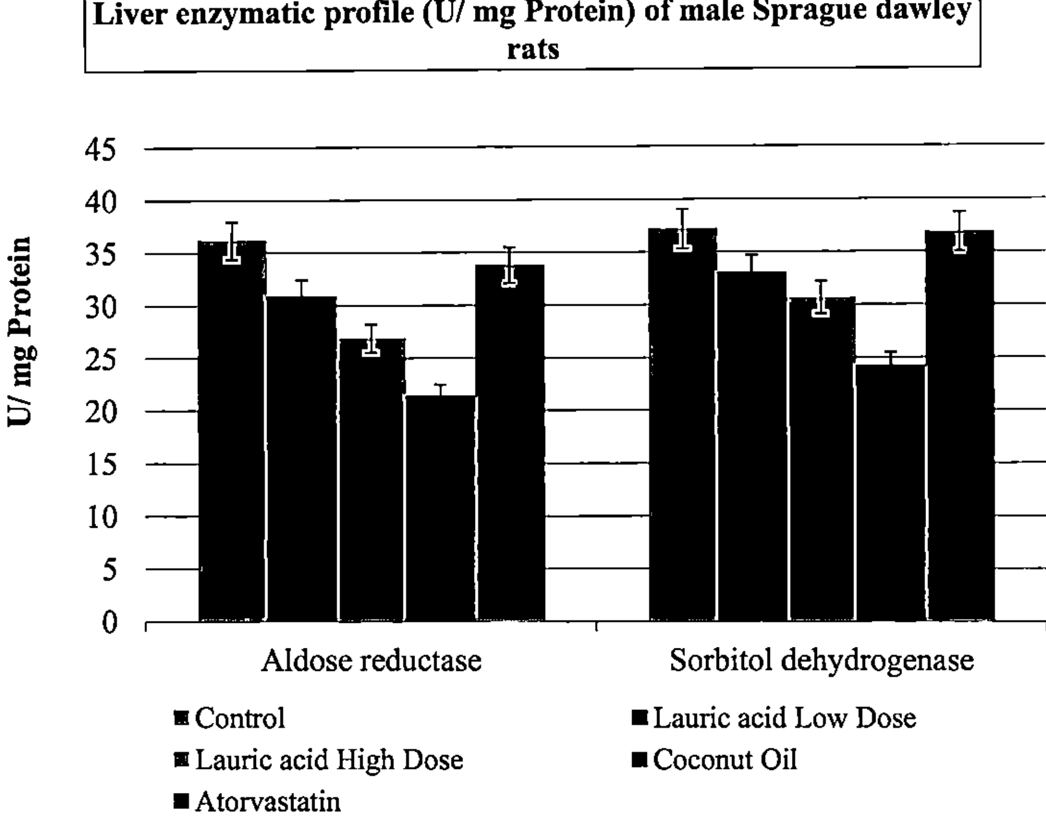


Figure 17: Liver enzymatic profile (U/ mg Protein) of male Sprague dawley rats showing the anti-diabetic property of coconut oil, lauric acid and atorvastatin

Among these three, coconut oil fed group recorded the maximum inhibition rate. These results give a clear idea that the consumption of coconut oil and the MCFAs can favour in lowering the diabetic complications.

Overall results from *in vivo* animal studies proved the anti-diabetic and hypolipidemic effects of coconut oil and lauric acid. Groups fed with 1 gm of lauric acid (LHD) was equalent to 2ml coconut oil fed groups since the lauric acid content in 2ml coconut oil is almost equal to 1gm lauric acid. The results obtained from *in vivo* experiments highlighted the fact that coconut oil was more effective in reducing hyperlipidemic and diabetic complications than lauric acid.

5.2.4 *In vitro* cytotoxicity analysis to validate anti-cancerous properties

In-vitro toxicity of lauric acid was performed on different cell lines like HCT 15, HepG2 and RAW 264.7. And it was found out that lauric acid is toxic to HCT 15, HepG2 and RAW 264.7 cell lines with IC_{50} (50 per cent of growth inhibition) values of 39, 46 and 36 μg/ml (Figure. 2, 3, and 4) concentrations. The toxicity study indicated that lauric acid had a dose dependent effect in arresting the cell growth of different cell lines. These results emphasize the anti-cancerous activity of lauric acid in inhibiting the mass multiplication of cancer cells and thereby lowering the severity of cancer disease.

5.2.5 Gene expression profile to validate anti-cancerous and anti-diabetic properties

IC_{50} value of lauric acid towards HCT 15 cell line was found to be 39 μg/ml. The results indicated that lauric acid was toxic and caused the death of half of cell population in the above concentration. Cell lines were treated with two different

sorbitol, the first step in polyol pathway of glucose metabolism. Glucose concentrations are often elevated in diabetics and aldose reductase has long been

concentration of lauric acid (30 and 50 μg/ml) at 24 hrs interval in order to study the gene expression profile of EGFR and Aldose reductase using qPCR.

5.2.5.1 Effect of lauric acid in the gene expression profile of EGFR identified for carcinoma

Mutations that lead to EGFR overexpression or up-regulation have been associated with a number of cancers, including lung cancer, anal cancer and colon cancer. These somatic mutations involving EGFR lead to its constant activation, which produces uncontrolled cell division (Normanno *et al.*, 2006). Recently many inhibitory drugs have been developed for EGFR which can suppress the over expression of this gene and there by inhibiting uncontrolled cell growth associated with it. In the present study, effect of lauric acid, on the expression of EGFR gene was analysed through q*RTPCR.* ΔΔCt value obtained for the cancer cell line HCT 15 when treated with lauric acid showed a positive inhibition in the expression of EGFR gene. Down regulation of this gene was more for the cells treated with 50 μg/ml of lauric acid (ΔΔCt= 0.46) compared to the cells treated with 30 μg/ml (ΔΔCt = 0.284) (Table 31 and Fig. 19). Figure 18 depicts the amplification plot and the Ct values obtained for EGFR gene in qPCR. This data highlights the importance of lauric acid, the natural compound in coconut, in suppressing the over expression of EGFR and there by controlling the outgrowth of disease to a certain limit. The present data was also supporting the *in silico* result obtained for lauric acid in cancer treatment.

5.2.5.2 Effect of lauric acid in the gene expression profile of Aldose reductase, involved in diabetes

qPCR analysis was also performed to analyse the gene expression profile of Aldose reductase in HCT 15 cell line treated with different concentrations of lauric acid. Aldose reductase catalyzes the NADPH-dependent conversion of glucose to sorbitol, the first step in polyol pathway of glucose metabolism. Glucose concentrations are often elevated in diabetics and aldose reductase has long been

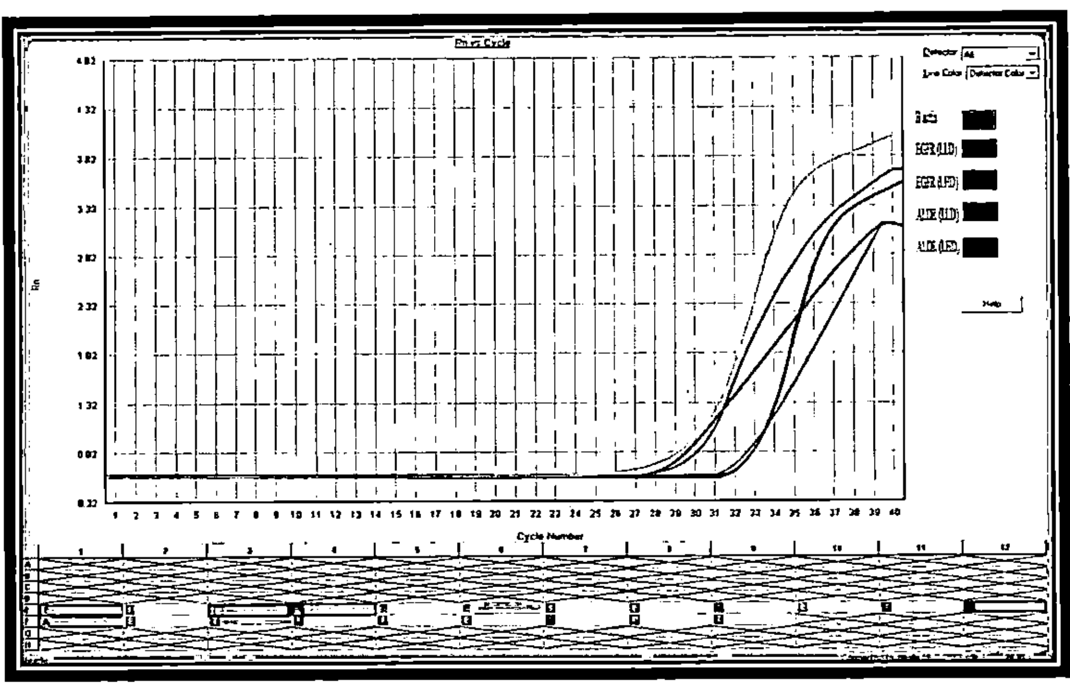


Figure 18: Amplification plot obtained from *qRTPCR* analysis for the genes Aldose reductase and EGFR against actin as reference gene

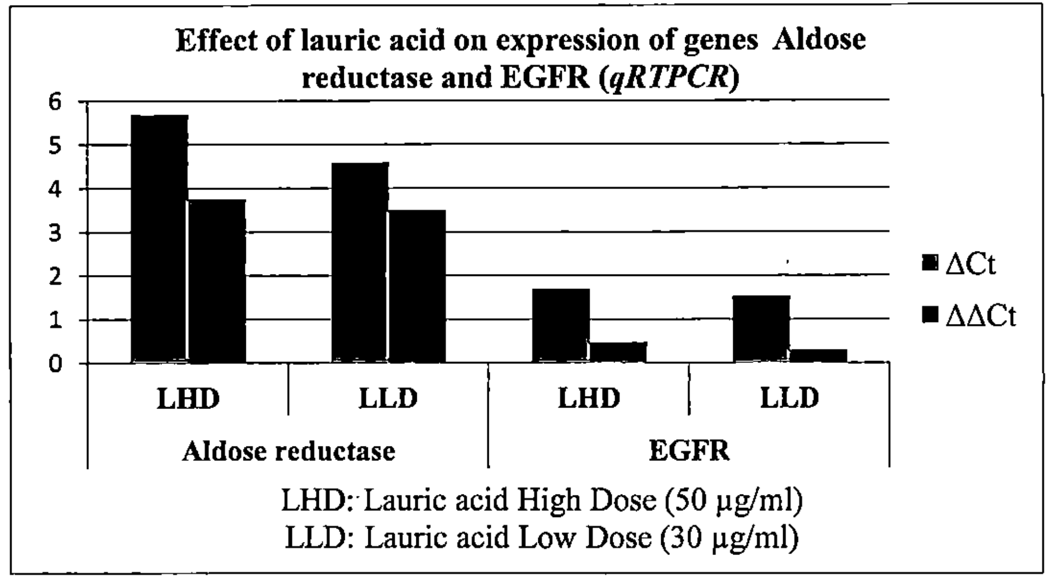


Figure 19: Effect of lauric acid on expression of genes Aldose reductase and EGFR (*qRTPCR*)

believed to be responsible for this type of diabetic complications. Figure 18 depicts the amplification plot and the Ct values obtained for gene Aldose reductase through qPCR. ΔΔCt values obtained for Aldose reductase indicated the positive effects of lauric acid in down regulating the gene expression and there by controlling the diabetic complications. In the *in silico* analysis Aldose reductase possessed good dock scores against lauric acid. Moreover it also expressed a positive tendency in reducing the diabetic complications while animal model experiments were done. The results obtained in q*RTPCR* also indicated a down- regulation in the expression of Aldose reductase gene in the cells treated with lauric acid. HCT 15 cell line treated with 50 µg/ml of lauric acid showed 3.75 fold decrease in the gene activity whereas the cell line treated with 30 µg/ml of lauric acid showed 3.49 fold decrease in Aldose reductase activity (Table 31 and Fig. 19). These results were emphasizing the importance of coconut phyto compounds in inhibiting the Aldose reductase protein and thereby lowering the diabetic complications.

Thus the present study highlights the significance of coconut phyto compounds through their interaction with different protein targets involved in various disorders like cancer, alzheimer's, diabetes and hyperlipidemics. Results of molecular docking highlights the fact that all the Medium Chain Fatty Acids (lauric, capric and caprylic acids) and polyphenols (gallic and caffeic acids) in coconut are effective in suppressing the disorders through their interaction with key enzyme/protein targets. Among the phyto compounds studied, the MCFA lauric acid was found to be superior in the inhibiting property. The wet lab analysis carried out for validation of *in silico* results confirmed the positive effect of lauric acid in inhibiting the protein targets involved in diseases like cancer, alzheimer's, diabetes and hyperlipidemics.

SUMMARY

SUMMARY

The study entitled "Molecular docking and validation of medicinal effects of coconut [*Cocos nucifera* L.]" was carried out in two stages viz. dry lab studies and wet lab studies during the period of 2013-15. Dry lab studies (*in silico* work) were carried out at Distributed Information Centre (DIC), Centre for Plant Biotechnology and Molecular Biology (CPBMB), College of Horticulture, Kerala Agricultural University with the objective to identify the medicinal properties of important phyto compounds in coconut by molecular docking, an *in silico* approach.

Wet lab analyses were carried out for validation of the results obtained from computational interactions. Works were taken up at Amala Cancer Research Centre, Thrissur with the approval of Animal Ethical Committee. These include *in vivo* and *in vitro* wet lab experiments.

In silico molecular docking was performed with the important protein targets involved in diseases such as cardiovascular disorders, cancer, alzheimer's, and diabetes. The phyto compounds of coconut such as lauric, capric, caprylic, caffeic and gallic acids were used as ligands. The interactions between target and ligand were analysed using different programmes in the commercial software Discovery studio, version 4. Compounds with positive interactions were further validated with *in vivo* animal models and *in vitro* cell line cultures. The interaction at gene expression level was also worked out through quantitative RTPCR.

The salient findings of the study are as follows:

1. Extensive literature survey revealed the compounds having major role in the medicinal properties of coconut. These include MCFAs such as lauric, capric, caprylic, myristic and polyphenols such as gallic and caffeic acids present in coconut oil.

5023–5028.

2. The medicinal properties of these phyto compounds were worked out through the level of inhibition of the protein targets involved in diseases such as cancer, alzheimer's, hyperlipidemics and diabetes. *In silico* molecular docking using the tool, Discovery studio version 4 was performed for the purpose. Commercial drugs identified for curing the selected diseases were also included in the study as ligands for comparison.
3. Coconut compounds retrieved through literature survey were first filtered using Veber and Lipinski's rule of five to study their drug likeness properties. Except myristic acid, all the other five ligands passed the Lipinski's rule.
4. Coconut phyto compounds and commercial drugs were docked with the protein targets identified for various disorders and the different dock scores such as binding energy, CDOCKER energy, CDOCKER interaction energy and hydrogen bond interactions were evaluated.
5. Seven proteins such as EGFR, CDK, DHFR, TS, VEGFR, ER and Bcl-xl confirmed to be involved in the occurance of cancer were selected as the targets for the docking analysis. The results indicated that all the five phyto compounds of coconut (lauric, capric, caprylic, caffeic and gallic acids) had an effective interaction with the seven targets identified for carcinoma. Among them, the most effective interaction was observed with the target Thymidylate synthase (TS).
6. Among the coconut phyto compounds, lauric acid recorded better interaction with all the seven targets selected for carcinoma with good dock scores and strong hydrogen bond interactions. Next to lauric, capric acid scored good interaction with all the targets.
7. Proteins such as APP, BACE 1 and BACE 2 were selected as the targets in the docking analysis for the neurogenic disorder, alzheimer's. Among the phyto compounds lauric acid was found most effective in inhibiting all the

targets, BACE 1, BACE 2 and APP and the dock scores recorded for lauric acid were far better than that of nonspecific commercial drugs analysed. Lauric acid also formed strong hydrogen bonds with the target.

8. Two protein targets selected for the *in silico* analysis of anti-diabetic properties of coconut phyto compounds were Aldose reductase and Dipeptidyl peptidase (DPP). The dock results were found superior for lauric acid against Aldose reductase, since the difference in CDOCKER energy and CDOCKER energy were almost nill. More over lauric acid possessed the best binding energy with a value of -203.45 kcal/ mol. SER 214 was identified as the active site amino acid residue forming strong hydrogen bond interactions.
9. *In silico* results indicated that natural compounds in coconut plays a significant role in reducing the cholesterol level by inhibiting the protein target HMGCo A reductase. The binding energy recorded for the coconut compounds against HMGCo A reductase ranged between -188.7 and -201.7 kcal/ mol. Lauric acid was identified as the best compound interacting most effectively with the target. LYS 722 and ARG 568 were the active site amino acid residues involved in the hydrogen bond interactions.
10. *In silico analysis* highlighted the inhibiting role of coconut based phyto compounds and lauric acid was identified as the best compound among the coconut phyto compounds studied.
11. Lauric acid was selected for validation of the results of *in silico* analysis through wet lab experiments. Wet lab analysis included cell line studies, animal model experiments and *qRTPCR.*
12. *In vivo* animal studies were carried out to validate the anti-diabetic and hypolipidemic nature of lauric acid and coconut oil. The results indicated that the lauric acid and coconut oil are effective in inhibiting the expression of key enzymes involved in the metabolic pathway leading to diabetes and

173727

hyperlipidemics. The lauric acid and coconut oil as such inhibited the synthesis of aldose reductase and sorbitol dehydrogenase, the enzymes involved in diabetic complications. They also inhibited HMG Co A reductase and lipoprotein lipase, enzymes having crucial role in cholesterol synthesis pathway. The hyperlipidemic and anti-diabetic properties of coconut oil were found better compared to lauric acid.

13. The anti-cancerous activity of coconut phyto compound was studied through *in vitro* cell line cultures. Lauric acid was found to be toxic in tumour cell lines HCT 15, HepG2 and RAW 564.7 with IC_{50} values of 39, 46 and 36 μg/ml respectively. The toxicity study indicated that lauric acid had a dose dependent effect in arresting the cell growth of different cell lines.
14. The gene expression profiles of Aldose reductase and EGFR in HCT 15 cell lines treated with 30 and 50 μg/ml of lauric acid were analysed through qPCR techniques. $\Delta\Delta$Ct value obtained for the cancer cell line HCT 15 when treated with lauric acid showed inhibition in the expression of EGFR gene. Down regulation of this gene was more for the cells treated with 50 μg/ml of lauric acid ($\Delta\Delta$Ct= 0.46) compared to the cells treated with 30 μg/ml ($\Delta\Delta$Ct= 0.284).
15. The results obtained in q*RTPCR* also indicated a down- regulation in the expression of Aldose reductase gene in the cells treated with lauric acid. HCT 15 cell line treated with 50 μg/ml of lauric acid showed 3.75 fold decrease in the Aldose reductase gene activity.
16. Overall results obtained from the *in silico*, *in vivo* and *in vitro* analysis emphasized the medicinal values of coconut oil and its major component, lauric acid in particular.
17. The present study could provide a strong scientific platform to highlight the importance of coconut oil and its phyto compounds for health benefits.

REFERENCES

REFERENCES

Agero, A. L. and Rowell, V. V. M. 2004. A randomized double-blind controlled trial comparing extra virgin coconut oil with mineral oil as a moisturizer for mild to moderate xerosis. *Dermatitis* **15**(3): 109-116.

Ahuja, S. and Ahuja, U. 2014. Coconut – History, Uses, and Folklore. *Asian Agri. Hist.* **18**(3): 221–248.

Akhila, S., Aleykutty, N. A., and Manju, P. 2012. Docking studies on *Peperomia pellucida* as antidiabetic drug. *Int. J. Pharmacy Pharma. Sci.* **4**(4): 1-3.

Allen, D. D., Caviedes, R., Cárdenas, A. M., Shimahara, T., Segura-Aguilar, J., and Caviedes, P. A. 2005. Cell lines as in vitro models for drug screening and toxicity studies. *Drug Dev. Ind. Pharma.* **31**(8): 757-768.

Aly, M. M., Shalaby, M. A., Attia, S. S., Sayed, S. H. E., and Mohmoud, S. S. 2013. Therapeutic effect of lauric acid, a Medium Chain Saturated Fatty Acid on *Giardia lamblia* in experimentally infected hamsters. *Parasitologists United J.* **6**(1): 89-98.

Ampasavate, C., Okonogi, S., and Anuchapreeda, S. 2010. Cytotoxicity of extracts from fruit plants against leukemic cell lines. *African J. Pharmacy Pharmacol.* **4**(1): 13-21.

Angadi, P. V. 2012. Monocarpin and doxycycline combination gel may be effective for treatment of *Herpes labialis*. *J. Evid. Based Dent. Pract.* **12**(4): 218-219.

Anitha, K., Gopi, G., Girish., and Kumar, P. S. 2013. Molecular docking study on dipeptidyl peptidase-4 inhibitors. *Int. J. Res. Dev. Pharmacy Life Sci.* **2**(5): 602-610.

Anneken, D., Sabine, B., Christoph, R., Fieg, G., Steinberne, U., and Westfechte, A. 2006. Fatty Acids: Ullmann's Encyclopedia of Industrial Chemistry

[on-line]. Available: http://onlinelibrary.wiley.com/doi/10.1002/1435600 7.a10_245.pub2/pdf. [1 Dec. 2014].

Arts, I. C. and Hollman, P. C. 2005. Polyphenols a disease risk in epidemiologic studies. *Am. J. Clin. Nutr.* **81**(1): 317-325.

Asokan, S., Kumar, R. S., Emmadi, P., Raghuraman, R., and Sivakumar, N. 2011. Effect of oil pulling on halitosis and microorganisms causing halitosis: A randomized controlled pilot trial. *Indian J. Dent. Res.* **29**(2): 90-94.

Assuncao, M. L., Ferreira, H. S., Santos, D. A. F., Cabral, C. R., and Florencio, T. M. 2009. Effects of dietary coconut oil on the biochemical and anthropometric profiles of women presenting abdominal obesity. *Lipids* **44**(7): 593-601.

Asthana, S., Agarwal, T., Banerjee, I., and Ray, S. S. 2014. *In silico* screening to elucidate the therapeutic potentials of asparagamine A. *Int. J. Pharmacy Pharma. Sci.* 6(3): 247-253.

Award, A. B. 1981. Effect of dietary lipids on composition and glucose utilization by rat adipose tissue. *J. Nutriticn.* **111**: 34 -39.

Barlow, D. J., Buriani, A., Ehrman, T., Bosisio, E., Eberini, I., and Hylands, P. J. 2012. *In-silico* studies in chinese herbal medicines' research: Evaluation of *in-silico* methodologies and phytochemical data sources, and a review of research to date. *J. Ethnopharmacol.* **140** (3): 526–534.

Berman, H. M. 2008. The Protein Data Bank: A Historical perspective. *Acta. Crystallographica* **64**: 88–95.

Blackburn, J. L., Kater, G., Mascioli, E. A., Kowalchuk, M., Babayan, V. K., and Bistrian, B. R. 1989. A revaluation of coconut oils effect on serum cholesterol and atherogenesis. *J. Phill. Med. Assoc.* **65**: 144-152.

Brooks, B. R., Brooks, C. L., Mackerell, A. D., Nilsson, L., Petrella, R. J., and Roux, B. 2009. CHARMM: The Bio-molecular simulation Program. *J. Comput. Chem.* **30**: 1545–1615.

Bucolo, G. and David, H. 1973. Quantitative determination of serum triglycerides by the use of enzymes. *Clin. Chem.* **19**(5): 476-82.

Bustin, S. A. 2002. Quantification of mRNA using real-time reverse transcription PCR (RT-PCR): trends and problems. *J. Mol. Endocrinol.* **29:** 23–39.

Carandang, E. V. 2008. Health benefits of virgin coconut oil. *Health* **31**(2): 8-12.

CDB [Coconut Development Board]. 2014. *All India final estimates of area and production of coconut 2013-14* [on-line]. Available: http://www.coconut board.nic.in/stat.htm [12 Sep. 2015].

Chan, E. and Elevitch, C. R. 2006. Species profiles for Pacific island agroforestry [on-line]. Available: http://www.traditionaltree.org [03 Nov. 2010]

Chandran, M., George, S., Santhalingam, K., Gangwar, P., and Krishnakumar, K. 2011. Molecular docking studies of 2α-Hydroxyursolic acid derivatives for hypercholesterolemia. *Int. J. PharmTech. Res.* **3**(3): 1576-1581.

Cohen, L. A., Thompson, D. O., Maeura, Y., and Weisburger, J. H. 1984. Influence of dietary medium- chain triglycerides on the development of N-methylnitrosourea- induced rat mammary tumor. *Cancer Res.* **44**: 5023–5028.

Cohen, L. A., Thompson, D. O., Maeura., Choi, K., Blank, M. E., and Rose, D. P. 1986. Dietary fat and mammary cancer. 1. Promoting effects of different dietary fats on N-nitrosomethylureainduced rat mammary tumorigenesis. *J. Natl. Cancer Inst.* **77** (1): 33-42.

Costantini, L. C., Barr, L. J., Vogel, J. L., and Henderson, S. T. 2008. Hypometabolism as a therapeutic target in Alzheimer's disease. *Neurosci.* 9(2): 16.

Dayrit, F. M. 2014. *Rediscovering the truth of the coconut: Curative properties of coconut oil* [on-line]. Available: http://www.philstar.com/science-andtechnology/2014/03/13/1300088/rediscovering-truth-coconut-curative-properties-coconut [10 September 2015].

deCremoux, F., PCoste, J., Cochand-Priollet, B., Le Galès, L. C., Cartier, I., Molinié, V., Labbé, S., Vacher-Lavenu, M. C., and Vielh, P. 2003. Cross sectional study of conventional cervical smear, monolayer cytology, and human papillomavirus DNA testing for cervical cancer screening. *British Med. J.* **5**(326): 733.

Dulloo, A. G., Fathi, M., Mensi, N., and Girardier, L. 1996. Twenty-four-hour energy expenditure and urinary catecholamines of humans consuming low-to-moderate amounts of medium-chain triglycerides: a dose-response study in a human respiratory chamber. *Eur. J. Clin. Nutr.* **50**(3): 152-158.

Enig, M. G. 2004. Coconut: In support of good health in the 21st Century [on-line]. Available: http://www.apcc.org.sg/special.htm [27 Aug 2015].

Expasy, 2016. *UniProtKB/Swiss-Prot protein knowledgebase release 2016_11 statistics* [on-line]. Available: http://web.expasy.org/docs/relnotes /relstat.html [1 Jan. 2016].

Farnsworth, N. R. and Soejarto, D. D. 1985. Potential consequences of plant extinction in the United States on the current and future availability of prescription drugs. *Econ. Bot.* **39**(3): 231–240.

Fauser, J. K., Matthews, G. M., Cummins, A. G., and Howarth, G. S. 2013. Induction of apoptosis by the medium-chain length fatty acid lauric acid in colon cancer cells due to induction of oxidative stress. *Chemotherapy* **59**(3): 214-224.

Fife, B. F. 2003. *The healing miracles of coconut oil.* Piccadilly Books: Colorado Springs, CO, USA, 521p.

Fife, B. F. 2005. *Coconut cures: preventing and treating common health problems with coconut.* Piccadilly Books: Colorado Springs, CO, USA, pp. 745–750.

Forastiere, A. and A, Burtness, B. A. 2007. Epidermal growth factor receptor inhibition in head and neck cancer: more insights, but more questions. *J. Clin. Oncol.* **25**: 2152-2155.

Gervajio, G. V. 2005. Fatty acids and derivatives from coconut oil. *Bailey's Industrial Oil Fat Products.* 6(6): 1-56.

Gond, D. S., Meshram, R. J., Jadhav, S. G., Wadhwa, G., and Gacche, R. N. 2013. *In silico* screening of chalcone derivatives as potential inhibitors of dihydrofolate reductase: Assessment using molecular docking, paired potential and molecular hydrophobic potential studies. *Drug Invention Today,* 31 May. 2013, pp. 182-191.

Grimwood, B. E., Ashman, F., Dendy, D. A. V., Jarman, C. G., Little, E. C. S., and Timmins, W. H. 1975. *Coconut Palm Products – Their processing in developing countries*. Rome, FAO, pp. 49–56.

Gupta, S., Pandey, A., Tyagi, A., and Mohan, C. G. 2010. Computational analysis of alzheimer's disease drug targets. *Curr. Inf. Pharma. Sci.* **11**(1): 1-9.

Hamid, M.A., Sarmidi, M. R., Mokhtar, T. H., Sulaiman, W. R. W., and Aziz, R.A. 2011. Innovative integrated wet process for virgin coconut oil production. *J. Applied Sci.* **11** (13): 2467.

Hariprasad, 2011. Molecular docking studies of plant derived compounds. *Asian J. Pharma. Clin. Res.* **5**(1): 87-88.

Harries, H.C. and Paull, R.E. 2008. *Cocos nucifera- Coconut.* In: Janick, J. and Paull, R.E. (Eds.) The Encyclopedia of Fruit and Nuts, Oxford University Press, USA, pp. 107-118.

Harvey, A. L. 2008. Natural products in drug discovery. *Drug Dis. Today* **13**(19): 894-901.

Hegde, B. M. 2006. Coconut oil – Ideal fat next only to mother's milk (Scanning Coconut's Horoscope). *J. Ind. Academy Clin. Med.* **7**(1): 16- 19.

Henderson, S. T., Vogel, J. L., Barr, L. J., Garvin, F., Jones, J. J., and Costantini, L. C. 2009. Study of the ketogenic agent AC-1202 in mild to moderate Alzheimer's disease: a randomized, double-blind, placebo-controlled, multicenter trial. *Nutr. Metabolism* **6**:31-32.

Hill, M. J. 1995. Diet and cancer: A review of the Scientific evidence. *Eur. J. Cancer Prev.* **4** (2): 3–42.

Hire, Kushal, K., and Dhale, D. A. 2012. Antimicrobial effect and *in silico* admet prediction of *Santalum album* L. *Int. J. Pharma. Bio Sci.* **3**(4): 727-734.

Hoshimoto, A., Suzuki, Y., Katsuno, T., Nakajima, H., and Saito, Y. 2002. Caprylic acid and medium-chain triglycerides inhibit IL-8 gene transcription in Caco-2 cells: Comparison with the potent histone deacetylase inhibitor trichostatin A. *British J. Pharmacol.* **136**: 280–286.

Hostmark, A. T., Spydevold, O., and Eilertseen, E. 1980. Plasma lipid concentration and liver output of lipoprotein in rats fed coconut fat or sunflower oil. *Artery* **7**: 367-383.

Iranloye, B., Oludare, G., and Olubiyi, M. 2013. Anti-diabetic and antioxidant effects of virgin coconut oil in alloxan induced diabetic male Sprague Dawley rats. *J. Diabetes Mellitus* **3**(4): 221-226.

Kabara, J. J., Swieczkowski, D. M., Conley, A. J., and Truant, J. P. 1972. Fatty acids and derivatives as antimicrobial agents. *Antimicrob. Agents Chemother.* **2**(1): 23-28.

Kalenkiewicz, A., Grant, B. J., and Yang, C. Y. 2015. Enrichment of druggable conformations from apoprotein structures using cosolvent-accelerated molecular dynamics. *Biol.* **4**: 344-366.

Kaplan, A., Rubaltelli, F. F., Hammerman, C., Vilei, M. T., Leiter, C., and Abramov, A. 1984. *Bilirubin*. The C.V. Mosby Co. St Louis, Toronto, Princeton, pp. 436, 650, 1238-1241.

Karimi, E., Oskoueian, E., Oskoueian, A., Omidvar, V., Hendra, R., and Nazeran, H. 2013. Insight into the functional and medicinal properties of *Medicago sativa* (Alfalfa) leaves extract. *J. Med. Plants Res*. 7(7): 290-297.

Kasai, M., Nosaka, N., Maki, H., Negishi, S., Aoyama, T., and Nakamura, M. 2003. Effect of dietary medium- and long-chain triacylglycerols (MLCT) on accumulation of body fat in healthy humans. *Asian Pacific J. Clin. Nut.* **12**: 151–160.

Kauntiz, H. 2001. Coconut oil consumption and coronary heart disease. *Philipp. J. Int. Med.* **30**: 165 – 171.

Kim, D. S., Sung, J. S., Shin, E. S., Ryu, J. S., Choi, I. K., Park, K. H., Park, Y., Kim, E. B., Park, S. J., and Kim, Y. H. 2008. Association of single nucleotide polymorphisms in Pim-1 gene with the risk of Korean lung cancer. *Cancer Res. Treat.* **40** (4): 190–196.

Kim, E. J., Cho, S. S., Jeong, Y., Park, K. C., Kang, S. J., Kang, E., Kim, S. E., Lee, K. H., and Na, D. L. 2005. Glucose metabolism in early onset versus late onset Alzheimer's Diseases: n SPM analysis of 120 patients. *Brain* **128**: 1790-1801.

Kimoto, Y., Tanji, Y., Taguchi, T., Sugimoto, T., Watanabe, T., Tsukamoto, F., Kim, S., Yoneda, K., Takamura, Y., Izukura, M., Shiba, E., and Takai, S. 1998. Antitumor effect of medium-chain triglyceride and its influence on the self-defense system of the body. *Cancer Detect. Prev.* **22**(3): 219-24.

Kindich, R., Florl, A. R., Jung, V., Engers, R., M¨uller, M., Schulz,W.A., and Wullich, B. 2005. Application of a modified real-time PCR technique for relative gene copy number quantification to the determination of the relationship between NKX3.1 loss and MYC gain in prostate cancer. *Clin. Chem.* **51**: 649-652.

Kitchen, D. B., Decornez, H., Furr, J. R., and Bajorath, J. 2004. Docking and scoring in virtual screening for drug discovery: methods and applications. *Nature Rev. Drug Discovery* **3**: 935-949.

Korn, E. D. 1959. The assay of lipoprotein lipase *in vivo* and *in vitro*. In: Glick, D. (ed.), *Methods of biochemical analysis*. New York Interscience Publishers. **7**: 145.

Kubiny, H. 1997. QSAR and 3D QSAR in drug design Part 1: methodology. *Drug Discovery Today* **2** (11): 457-467.

Kumar, D. R., Lakshmi, P. S., Saravani, N., and Marimuthu, S. 2012. *In silico* molecular docking studies on porcine pancreatic phospholipase A2 against plant extracts of phenolic inhibitors. *Int. J. Res. Biomed. Biotechnol.* **2**(3): 8-16.

Kumar, M. K. 2008. *Krishi Gita (Agricultural Verses).* Agri-History Bulletin No. 7. Asian Agri-History Foundation, Secunderabad, India, 111pp.

Kurup, P. A. and Rajmohan, T. 1994. Consumption of coconut oil and coconut kernel and the incidence of atherosclerosis. Proceedings of symposium on coconut and coconut oil in human nutrition, 27 March 1994. Coconut Development Board, Kochi, India, pp. 35-59.

Lewington S, Whitlock G, Clarke R, Sherliker P, Emberson J, Halsey J, Qizilbash N, Peto R, and Collins R. 2007. Blood cholesterol and vascular mortality by age, sex, and blood pressure: a meta-analysis of individual data from 61 prospective studies with 55,000 vascular deaths. *Lancet* **370** (9602): 1829–1839.

Lim, F. P. K., Bongosia, L. F. G., Yao, N. B. N., and Santiago, L. A. 2014. Cytotoxic activity of the phenolic extract of virgin coconut oil on human hepatocarcinoma cells (HepG2). *Int. Food Res. J.* **21**(2): 729-733.

Lindeberg, S. and Lundh, B. 1993. Apparent absence of stroke and ischaemic heart disease in a traditional Melanesian island: a clinical study in Kitava. *J. Intern. Med.* **233**(3): 269-275.

Lipinski, C.A., Lombardo, F., Dominy, B. W., and Feeney, P. J. 2001. Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. *Adv. Drug Deliv.* **46**(1-3): 3-26.

Liu, Y. M. 2008. Medium-chain triglyceride (MCT) ketogenic therapy. *Epilepsia* **8**: 33-36.

Livak, K. J. and Schmittgen, T. D. 2001. Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta C}$ T method. *Methods* **25**, 402–408.

Loyer, P., Trembley, J., Katona, R., Kidd, V., and Lahti, J. 2005. Role of CDK/cyclin complexes in transcription and RNA splicing. *Cell Signal* **17**(9): 1033-1051.

Lund, J. D. D. 2005. The Estrogen Receptor. Bachelor's Thesis, Department of Chemistry, University of Aarhus, Denmark, pp: 1- 43.

Lutz, M. K. 2003. Putting nature back into drug discovery. *Nature Biotechnol.* **21**(3): 602-604.

Mala, S. K., Priya, A. P., Bincy, B., and Nazeem, P. A. 2015. Bioactive compounds to target anti apoptotic proteins- bcl 2 and bcl xl an *in silico* approach. *Int. J. Pharma. Sci. Res.* **6**(7): 2320-5148.

Malongil, B., Reena., Sunki, R., Reddy, Y., Belur, R., and Lokesh. 2009. Changes in triglyceride molecular species and thermal properties of

blended and interesterified mixtures of coconut oil or palm oil with rice bran oil or sesame oil. *Eur. J. Lipid Sci. Tech.* **111**: 346-357.

Mandal, M.D. and Mandal, S. 2011. Coconut (*Cocos nucifera* L: Arecaceae): In health promotion and disease prevention. *Asian Pacific J. Tropc. Med.* pp: 241-247.

Mansor, T. S. T., CheMan, Y. B., Shuhaimi, M., Afiq, A. M. J., and Ku-Nurul, F. K. M. 2012. Physicochemical properties of virgin coconut oil extracted from different processing methods. *Int. Food Res. J.* **19** (3): 837-845.

Mathew, A. J. and Raj, N. N. 2009. Docking studies on anticancer drugs for breast cancer using hex. In: Ao, S. I., Castillo, O., Douglas, C., Feng, D. D., and Lee, J. A. (eds.), *Proceedings of the International multiconference of Engineers and Computer Scientists.* 18 – 20 March, 2009, Hong Kong, International Association of Engineers, pp.1-578.

Mathi, P., Nikhil, K., Ambatipudi, N., Roy, P., Bokka, V. R., and Botlagunta, M. 2014. *In-vitro* and *in-silico* characterization of *Sophora interrupta* plant extract as an anticancer activity. *Bioinformation* **10**(3): 144–151.

Mcclemon, F. J., Yancy, W. S., Eberstein, J. A., Atkins, R. C., and Westman, E. C. 2007. The effects of a low-carbohydrate ketogenic diet and a low-fat diet on mood, hunger, and other self-reported symptoms. *Obesity* **15**(1): 182-187.

Mensink, R. P., Zock, P. L., Kester, A. D. M., and Katan, M. B. 2003. Effects of dietary fatty acids and carbohydrates on the ratio of serum total to HDL cholesterol and on serum lipids and apolipoproteins: a meta-analysis of 60 controlled trials. *Amer. J. Cli. Nut.* **77**(5): 1146–1155.

Mishra, K.P., Sharma, N., Diwaker, D., Ganju, L., and Singh, S. B. 2013. Plant derived antivirals: a potential source of drug development. *J. Virol. Antivir.* **2**: 2-9.

Mukhtar, M., Arshad, M., Ahmad, M., Pomerantz, R. J., Wigdahl, B., and Parveen, Z. 2008. Antiviral potentials of medicinal plants. *Virus Res.* **131**: 111-120.

Naito, H. K. 1984. *Cholesterol.* The C.V. Mosby Co. St Louis. Toronto. Princeton, pp. 1194-11206, 437.

Nandakumar, T. B. 1998. Recent development in coconut industry in India with special reference to Kerala. *Indian Coconut J.* pp.6-8.

NCBI [National Center for Biotechnology Information]. 2015. *GenBank release notes* [on-line]. Available: http://www.ncbi.nlm.nih.gov/nuccore [3 Aug. 2015]

Neal, E. G., Schwartz, H. R., Lawson, M. S., Edwards, N., Fitzsimmons, G., Whitney, A., and Cross, J. H. 2008. The ketogenic diet for the treatment of childhood epilepsy: a randomised controlled trial. *Lancet Neurol. J.* 7(6): 500-506.

Nebeling, L. C., Miraldi, F., Shurin, S. B., and Lerner E. 1995. Effects of a ketogenic diet on tumor metabolism and nutritional status in pediatric oncology patients: two case reports. *J. Am. Coll. Nutr.* **4**(2): 202-208.

Nevin, K. G. and Rajamohan, T. 2004. Beneficial effects of virgin coconut oil on lipid parameters and in vitro LDL oxidation. *Clin. Biochem.* **37**(9): 830-835.

Nevin, K. G. and Rajamohan, T. 2010. Effect of topical application of virgin coconut oil on skin components and antioxidant status during dermal wound healing in young rats. *Skin Pharmacol. Physiol.* **23** (6): 290-297.

Newport, M. T. 2011. *Alzheimer's Disease: What If There Was a Cure?* [on-line]. Basic Health Publication, Dunham. Available: http://www.coconut ketones.com.

Nogrady, B. 2008. Coconut good fat or bad? [on-line]. Available: http://www.abc.net.au/health/thepulse/stories/2008/09/25/2372372.htm [22 Sep. 2015].

Normanno, N., Luca, D. A., Bianco, C., Strizzi, L., Mancino, M., Maiello, M. R., Carotenuto, A., Feo, D. G., Caponigro, F., and Salomon, D. S. 2006. Epidermal growth factor receptor (EGFR) signaling in cancer. *Gene* **366**(1): 2-16

Ogbolu, D. O., Oni, A. A., Daini, O. A., and Oloko, A. P. 2007. *In vitro* antimicrobial properties of coconut oil on candida species in Ibadan, Nigeria. *J. Med. Food* **10**(2): 384-387.

Papamandjaris, A. A., MacDougall, D. E., and Jones, P. J. 1998. Medium chain fatty acid metabolism and energy expenditure: obesity treatment implications. *Life Sci.* **62**(14): 1203-1215.

Patel, S., Gheewala, N., Suthar, A., and Shah, A. 2009. *In-vitro* cytotoxicity activity of *Solanum nigrum* extract against *Hela* cell line and *vero* cell line. *Int. J. Pharmacy Pharma. Sci.* **1**(1): 38-46.

Peters, G. J., Backus, H. H., Freemantle, S., van Triest, B., Codacci-Pisanelli, G., van der Wilt, C. L., Smid, K., Lunec, J., Calvert, A. H., Marsh, S., McLeod, H. L., Bloemena, E., Meijer, S., Jansen, G., van Groeningen, C. J., and Pinedo, H. M. 2002. Induction of thymidylate synthase as a 5-fluorouracil resistance mechanism. *Biochem. Biophys. Acta.* **1587** (2-3): 194–205.

Pfaffl, M. W. 2001. . A new mathematical model for relative quantification in real-time RT–PCR. *Nucl. Acids Res.* **29**(9): 8-12.

Pratheepa, M. 2012. GC-MS and *in silico* analysis of *Cleistanthus collinus* for its activity against cancer. *Drug discovery* **1**: 23-26.

Priller, C., Bauer, T., Mitteregger, G., Krebs, B., Kretzschmar, H. A., and Herms, J. 2006. Synapse formation and function is modulated by the amyloid precursor protein. *J. Neurosci.* **26** (27): 7212–7221.

Prior, I. A., Davidson, F., Satmond, C. E., and Ezochans, K. 1981. Cholesterol, coconuts and diet on polynesion atolls: a natural experiment: the Pukpuka and Tokelan Island stdues. *Am. J. Clin. Nutrition.* **34**: 1552 – 1561.

Pugazhendhi, D. and Umamaheswari, S. 2013. *In silico* methods in drug discovery. *Int. J. Adv. Res. Comput. Sci. Software Eng.* **3**(5): 680. Available: www.ijarcsse.com [8 Aug. 2014].

Raj, P. S., Prathapan, A., Sebastian, J., Antony, A. K., Riya, M. P., Rani, M. R., Biju, H., Priya, S., and Raghu, K. G. 2014. Parmotrema tinctorum exhibits antioxidant, antiglycation and inhibitory activities against aldose reductase and carbohydrate digestive enzymes: An in vitro study. *Nat. Product Res.* **28** (18): 1480–1484.

Rao, A. V. and Ramakrishnan, S. 1975. Indirect assessment of hydroxymethylglutaryl-CoA reductase (NADPH) activity in liver tissue. *Clin. Chem.* **21**(10): 1523–1525.

Reddy, A. B., Ramana, K. V., Srivastava, S., Bhatnagar, A., and Srivastava, S. K. 2008. Aldose reductase regulates high glucose-induced ectodomain shedding of tumor necrosis factor (TNF)-alpha via protein kinase C-delta and TNF-alpha converting enzyme in vascular smooth muscle cells. *Endocrinol.* **150**(1): 63-74.

Reddy, B. S. and Maeura, Y. 1984. Tumor promotion by dietary fat in azoxymethane-induced colon carcinogenesis in female F344 rats: influence of amount and source of dietary fat. *J. Nat. Canc. Inst.* **72**: 745–750.

Reddy, G. B., Satyanarayana, A., Balakrishna, N., Ayyagari, R., Padma, M., Viswanath, K., and Petrash, J. M. 2008. Erythrocyte aldose reductase activity and sorbitol levels in diabetic retinopathy. *Mol. Vis.* **14**: 593–601.

Reger, M. A., Henderson, S. T., Hale, C., Cholerton, B., Baker, L. D., Watson, G. S., Hyde, K., Chapman, D., and Craft, S. 2004. Effects of beta-hydroxybutyrate on cognition in memory-impaired adults. *Neurobiol. Aging* **25**(3): 311-314.

Reichhard, T. 1999. It's sink or swim as a tidal wave of data approaches. *Nature* **399**(6736): 517-520.

Rele, A. S. and Mohile, R. B. 2003. Effect of mineral oil, sunflower oil, and coconut oil on prevention of hair damage. *J. Cosmet. Sci.* **54**(2): 175-192.

Röhrig, U. F., Awad, L., Grosdidier, A., Larrieu, P., Stroobant, V., Colau, D., Cerundolo, V., Simpson, A. J., Vogel, P., Eynde, V. B. J., Zoete, V., and Michielin, O. 2010. Rational design of indoleamine 2,3-dioxygenase inhibitors. *J. Med. Chem.* **53**(3): 1172-89.

Sahu, R. and Shukla, N. 2014. *In- silico* analysis of different plant protein and their essential compound with sulfonylurea binding protein of cells of Homo sapiens for curing diabetes mellitus type ii disease. *Eur. Chem. Bull.* 3(6): 568-576.

Sanghani, H. V., Ganatra, S. H., and Pande, R. 2012. Molecular – Docking studies of potent anticancer agent. *Comput. Sci. Syst. Biol.* **5**(1): 12-15.

Scalfi, L., Coltorti, A., and Contaldo, F. 1991. Postprandial thermogenesis in lean and obese subjects after meals supplemented with medium-chain and long-chain triglycerides. *Am. J. Clin. Nutr.* **53**(5): 1130-1133.

Seaton, T. B., Welle, S. L., Warenko, M. K., and Campbell, R. G. 1986. Thermic effect of medium-chain and long-chain triglycerides in man. *Am. J. Clin. Nutr.* **44**(5): 630-634.

Shariq, B., Zulhabri, O., Hamid, K., Sundus, B., Mehwish, H., Sakina, R., Jiyauddin, K., Kaleemullah, M., Sameer, A. D., and Rasha, S. 2015. Evaluation of anti-atherosclerotic activity of virgin coconut oil in male

wistar rats against high lipid and high carbohydrate diet induced atherosclerosis. *J. Pharama. Biosci.* **3**(2): 10-14.

Sharma G. 2014. *The miraculous health benefits of coconut oil* [on-line]. Weight Management Expert. Available: http://food.ndtv.com/opinions/the-miraculous-health-benefits-of-coconut-oil-707008 [1 Jan. 2015].

Sharma, V. and Sarkar, I. N. 2012. Bioinformatics opportunities for identification and study of medicinal plants. *Brief Bioinform.* **14**(2): 238-250.

Shruthila, N., Narayan, P., Kumar, D. J., and Nagendra, H. G. 2014. Docking studies of coconut oil components with amyloid beta peptide fragments. *J. Neurol. Neurophysiol.* **5**(5): 1-6.

Siri-Tarino, P. W., Sun, Q., Hu, F. B., and Krauss, R. M. 2010. Meta-analysis of prospective cohort studies evaluating the association of saturated fat with cardiovascular disease. *Am. J. Clin. Nutr.* **91**(3): 535-546.

Sirtori, C. R. 2006. HDL and the progression of atherosclerosis: new insights. *Eur. Heart J.* **8**: 4-9.

Sivashanmugam, T., Muthukrishnan, S., Umamaheswari, M., Asokkumar, K., Subhadradevi, V., Jagannath, P., and Madeswaran, A. 2013. Discovery of potential cholesterol esterase inhibitors using *in silico* docking studies. *Bangladesh. J. Pharmacol.* **8**: 223-229.

Srivastava, V., Gupta, S. P., Siddiqi, M. I., and Mishra, B. N. 2010. Molecular docking studies on quinazoline antifolate derivatives as human thymidylate synthase inhibitors. *Bioinformation* **4**(8): 357-365.

Stahlberg, A., Zoric, N., Aman, P., and Kubista, M. 2005. Quantitative real time PCR for cancer detection: the lymphoma case. *Mol. Diagn.* **5**(2): 221-230.

Stubbs, R. J. and Harbron, C. G. 1996. Covert manipulation of the ratio of medium- to long-chain triglycerides in iso energetically dense diets: Effect

on food intake in ad libitum feeding men. *Int. J. Obes. Relat. Metab. Disord.* **20**(5): 435-444.

Sweetman. 2009. Cardiovascular drugs. In: Sean, C. (ed.), *Martindale: the complete drug reference* (36th Ed.). London: Pharmaceutical Press, pp. 1155–1434.

Taylor, L. N. D. 2000. Plant based drugs and medicines [on-line]. Available: http://www.rain-tree.com/plantdrugs.htm#.VlbEQVKzmwU [16 Sep. 2014].

Teoh, S. L., Latiff, A. A., and Das, S. 2010. Histological changes in the kidneys of experimental diabetic rats fed with *Momordica charantia* (bitter gourd) extract. *Rom. J. Morphol. Embryol.* **51**(1): 91-95.

Thampan, P. K. 1981. *Hand Book of coconut palm.* Oxford and IBH New Delhi publishers, 557p.

Thampan, P.K. 1994. *Facts and fallacies about coconut oil.* Asian and Pacific Coconut Community, 8p.

Thijssen, M.A. and Mensink. R. P. 2005. Fatty acids and atherosclerotic risk. In: Eckardstein, A. V. (ed.), *Atherosclerosis: diet and drugs*. Springer, pp. 171–172.

Tiwari, M. K., Singh, R., Singh, R. K., Kim, I. W., and Lee, J. K. 2012. Computational approaches for rational design of proteins with novel functionalities. *Compt. Str. Biotech. J.* **2**: 1- 10. Available: http://www.ncbi .nlm.nih.gov/pmc/articles/PMC3962203/ [2 Mar. 2014].

Tsuji, H., Kasai, M., Takeuchi, H., Nakamura, M., Okazaki, M., and Kondo, K. 2001. Dietary medium- chain triglycerides suppress body fat accumulation in a double blind, controlled trial in healthy men and women. *J. Nutr.* **131**: 2853-2859.

Veech, R. L., Chance, B., Kashiwaya, Y., Lardy, H. A., and Cahill, G. C. 2001. Ketone bodies, potential therapeutic uses. *IUBMB Life* **51**(4): 241-247.

Veeresham, C. 2012. Natural products derived from plants as a source of drugs. *J. Adv. Pharm. Technol.* **3**(4): 200–201.

Vijith, V. S., Harikrishnan, K. V., Krishnapriya, S., Varma, V., Jose, A., Ramani, P., Kumar, E. P., and Namboori, K. P. K. 2013. Design of novel drug molecules for the treatment of breast cancer. *Int. J. Pharma. Sci.* **20**(2): 113-117.

Vysakh, A., Ratheesh, M., Rajmohan, T. P., Pramod, C., Premlal, S., and Kumar, G. B. 2014. Polyphenolics isolated from virgin coconut oil inhibits adjuvant induced arthritis in rats through antioxidant and anti-inflammatory action. *Int. Immunopharmacol.* **20**(1): 1-8.

Wallingford, J. B., Liu, K. J., and Zheng, Y. 2010. Xenopus. *Curr. Bio.* **20**(6): 263-264.

Woodroof, J. G. 1970. Coconuts: production, processing, products. *Oxford J. Med. Health* **32**(2): 200-209.

Wu, G., Robertson, D.H., Brooks, C.L., and Vieth, M. 2003. Detailed analysis of grid-based molecular docking: A case study of CDOCKER—A CHARMm-based MD docking algorithm. *J. Compt. Chem.* **24**(13): 1549-1562.

Wymelbeke, V. V., Himaya, A., Sylvestre, L. J., and Fantino, M. 1998. Influence of medium-chain and long-chain triacylglycerols on the control of food intake in men. *Am. J. Clin . Nutr.* **68**(2): 226-234.

Zahedani, M. R., Alinejad, N., Zadeh, S. M. M. A., and Mazloom, Z. 2015. Comparison of the effects of edible oils: Rice bran, grape seed, and canola on serum lipid profile and paraoxonase activity in hyperlipidemic rats. *Int. Cardiovasc. Res. J.* **9**(1): 28-33.

Zhao, J., Jiang, T., Li, H., Zhang, Y., and Zhang, N. 2012. Aldose reductase regulates TNF-α-induced inducible nitric oxide synthase expression in human mesangial cells. *Mol. Biol. Rep.* **39**(2): 1815-1822.

ANNEXURE

ANNEXURE I

Coconut compounds used as ligands in the molecular docking analysis and their PubChem ID

Sl. No.	Coconut compounds	PubChem ID	Structure
1	Lauric acid	3893	
2	Capric acid	2969	
3	Caprylic acid	379	
4	Myristic acid	11005	
5	Gallic acid	370	
6	Caffeic acid	689043	

ANNEXURE II

List of gene specific primers used in *qRTPCR* analysis

Sl. No.	Name of primers	Nucleotide sequence		Number of bases
1	Epidermal Growth Factor Receptor (EGFR)	F	5'GAGACGAGAACTGCCAGAA3'	19
		R	5'GTAGCATTTATGGAGAGTC3'	19
4	Aldose reductase	F	5'AAAGGAGCCTGCCAGAAGAC3'	20
		R	5'TTCACCAGCCCTTCATCCAC3'	20

MOLECULAR DOCKING AND VALIDATION OF MEDICINAL EFFECTS OF COCONUT (*Cocos nucifera* L.).

By

DEVI LEKSHMI S

(2013-11-192)

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

VELLANIKKARA, THRISSUR - 680 656

KERALA, INDIA

2016

ABSTRACT

Medicinal properties of coconut are mainly attributed by the coconut oil which is enriched with MCFAs like lauric acid, myristic acid, capric acid and caprylic acid. Coconut oil contains 92 per cent saturated fatty acids, of which 72 per cent are Medium Chain Fatty Acids (MCFAs). Among the MCFAs, lauric acid is the most abundant (52 %) and potent compound. Polyphenols like gallic acid and caffeic acid also have role in promoting the health benefits of coconut oil. Controversy in consumption of coconut oil with respect to its beneficial or hazardous effects to health exists for a long time. The study entitled 'Molecular docking and validation of medicinal effects of coconut (*Cocos nucifera* L)' was aimed to analyze the medicinal effects of important components in coconut through *in silico* analysis and its validation through wet lab studies. Effect of various components to inhibit or activate the targets involved in cardiovascular disorders, cancer, alzheimer's, diabetes etc. were analyzed through molecular docking and the positive effects validated.

In silico molecular docking was performed with important protein targets identified for the selected diseases and the phyto compounds of coconut as ligands using the commercial software Discovery studio, version 4 at Distributed Information Center (DIC), College of Horticulture. Wet lab experiments were done through *in vivo* animal model experiments and *in vitro* cell line cultures in collaboration with Amala Cancer Research Centre, Thrissur. Gene expression studies were conducted for validating the anti-cancerous and anti-diabetic properties through *qRTPCR*.

Phyto compounds of coconut such as lauric, capric, caprylic, gallic and caffeic acid exhibited an effective interaction with the seven protein targets of cancer such as EGFR, CDK, DHFR, TS, VEGFR, ER and Bcl-xl and the best interaction was shown with the Thymidylate synthase (TS). Among the coconut phyto compounds lauric acid recorded better interaction against TS. In case of alzheimer's, lauric acid was found more effective in inhibiting the targets, BACE

1, BACE 2 and APP. The dock results were found superior for lauric acid against the protein Aldose reductase, identified for diabetes and HMG Co A reductase, identified for hyperlipidemics.

Lauric acid recorded better results in *in silico* molecular docking analysis among the coconut phyto compounds selected. The medicinal properties of lauric acid were further validated through wet lab experiments. *In vivo* animal studies proved the anti-diabetic and hypolipidemic nature of lauric acid and coconut oil. They were effective in inhibiting the synthesis of aldose reductase and sorbitol dehydrogenase, enzymes involved in diabetic complications. Also the level of HMG Co A reductase and lipoprotein lipase enzyme activity were reduced when treated with coconut oil and lauric acid. These enzymes have a crucial role in cholesterol synthesis path way. The cell line toxicity studies indicated the anti-cancerous activity of lauric acid and its dose dependent effect in arresting the tumor growth. Gene expression analysis using q*RTPCR* exhibited a down-regulation of Aldose reductase gene, identified for diabetes and EGFR gene, for cancer when the cells were treated with lauric acid.

Overall results obtained from the molecular docking analysis, animal studies, cell line cultures and gene expression assays emphasized the medicinal values of important phyto compounds in coconut oil. Thus the present study provides a strong scientific backing to highlight the health benefits of coconut oil.

Abbreviations

EGFR- Epidermal Growth Factor Receptor

CDK- Cylin Dependent Kinase

DHFR- Dihydro Folate Reductase

VEGFR- Vascular Endothelial Growth Factor Receptor

ER- Estrogen Receptor

Bcl-xl- B cell lymphoma cancer APP- Amyloid precursor protein

BACE 1& 2 – Beta Secretase 1 and 2