IDENTIFICATION AND ISOLATION OF POLYPHENOLS AS ANTIGLYCATIVE AGENTS FROM SOME SELECTED INDIAN FRUIT SEEDS

A Project Report

Submitted by

SHAKTHI DEVE A

(Reg. No. 1120203013)

In partial fulfillment of the requirements for the award of the degree of

MASTER OF TECHNOLOGY

Faculty of BIOTECHNOLOGY

KUMARAGURU COLLEGE OF TECHNOLOGY

(An Autonomous Institution Affiliated to Anna University, Chennai)

COIMBATORE -641 049

May 2013

IDENTIFICATION AND ISOLATION OF POLYPHENOLS AS NOVEL ANTIGLYCATIVE AGENTS FROM SOME SELECTED INDIAN FRUIT SEEDS

A PROJECT REPORT

Submitted by

SHAKTHI DEVE A
(Reg No 1120203013)

in partial fulfillment for the requirement of award of the degree

of

MASTER OF TECHNOLOGY

in

BIOTECHNOLOGY

Department of Biotechnology

KUMARAGURU COLLEGE OF TECHNOLOGY, COIMBATORE 641049

(An Autonomous institution to Anna University, Chennai)

May 2013

BONAFIDE CERTIFICATE

Certified that this project report entitled **IDENTIFICATION AND ISOLATION OF POLYPHENOLS AS ANTIGLYCATIVE AGENTS FROM SOME SELECTED INDIAN FRUIT SEEDS** is the bonafide work of **Ms. SHAKTHI DEVE A (Reg. No. 1120203013)** who carried out the research under my supervision. Certified further that to the best of my knowledge, the work reported herein does not form part of any other project report or dissertation, on the basis of which, a degree or award was conferred on an earlier occasion on this or any other candidate.

Dr. T. SATHISHKUMAR
Supervisor
Assistant Professor (SrG)
Department of Biotechnology,
Kumaraguru College of Technology,
Chinnavedampatti,
Coimbatore - 641 049

Dr. A. MANICKAM
Professor and Head
Department of Biotechnology,
Kumaraguru College of Technology,
Chinnavedampatti,
Coimbatore - 641 049

ABSTRACT

Diabetes mellitus is a chronic metabolic disorder characterized by increased blood glucose level (Hyperglycemia). It has become an epidemic disease in the 21st century where, India leads the world with largest number of diabetic subjects. Non-enzymatic glycosylation (glycation) is severe form of diabetes, occurs between reducing sugar and proteins which results in the formation of Advanced Glycation End products (AGEs), that leads to other complicated secondary diseases. In this context, *Mangifera indica* (Mango), *Syzygium cumini* (Jambul), *Vitis vinifera* (Grapes), *Citrus sinensis* (Orange), *Artocarpus heterophyllus* (Jackfruit), *Manilkara zapota* (Sapodilla) seeds were utilized for the present investigation inorder to identify and isolate the polyphenols as novel antiglycative agents. Different extraction methods (shake flask, centrifugation and pressurized hot water) using various extractants (organic solvents, hot water and pressurized hot water) revealed that pressurized hot water extract of Jackfruit possess high antiglycation activity (87.52 %) and Orange showed low antiglycation activity (74.79 %). Factors such as hexane to heptane ratio (v/v), time (min) and solid to liquid ratio (g/ml) were optimized using Response Surface Methodology (RSM) in the extraction of polyphenols from Orange seeds. The results revealed that 1:15 solvent ratio, 6 minutes and 1:20 Solid to liquid ratio possess an optimal antiglycation activity (89.79 %). The TLC analysis revealed the presence of polyphenols. The LC-PDA-MS analysis of PTLC (Preparative Thin Layer Chromatography) isolates of Jackfruit seed extracts showed the presence of compounds like Quercetin (301.2), 4-hydroxy phenyl acetic acid

(149.0), 4-hydroxybenzaldehyde (121.0), Rhamnosyl-dihexosyl Quercetin sulphate (853.0), rutin (613.4), Diosmetin (300) and luteolin (283.0).

KEYWORDS: Diabetes mellitus, Response Surface Methodology, *Artocarpus heterophyllus* (Jackfruit), *Citrus sinensis* (Orange), Qurcetin.

ACKNOWLEDGEMENT

I express my humble gratitude to my guide **Dr.T.Sathishkumar,** Assistant Professor (SrG), Department of Biotechnology, Kumaraguru College of Technology, Coimbatore, for his constructively critical guidance, utmost motivation, valuable advice, untiring support, timely suggestion, constant encouragement, relentless patience, continued keen interest and inspiration throughout the study.

I wish to express my deep sense of reverential gratitude to **Dr.S.Ramachandran,** Principal, Kumaraguru College of Technology, Coimbatore, for providing the facilities to conduct this study.

I express my humble gratitude to **Dr.A.Manickam,** Head of the Department, Department of Biotechnology, Kumaraguru College of Technology, Coimbatore, for giving me kind permission to carry out my project.

I solemnly submit my humble thanks to **Dr.N.Saraswathy**, Project coordinator, Associate Professor, Department of Biotechnology, Kumaraguru College of Technology, Coimbatore, for facilitating conditions to carry out the research work smoothly.

I owe a lot to my review committee members **Dr.V.Stephen Rapheal**, Associate professor and **Dr.K.Kumaresan,** Assistant Professor (SrG), Department of Biotechnology, Kumaraguru College of Technology for helping me with their valuable ideas and suggestions.

I sincerely thank all the **teaching and non-teaching staff members** of the Department of Biotechnology, Kumaraguru College of Technology, Coimbatore, for being supportive and understanding.

I also extend my sincere thanks to SAIF (Sophisticated Analytical Instrument Facility), IIT BOMBAY for LC-MS analysis.

Lastly, I would like to thank my friends and family for supporting me and encouraging me throughout the project.

(SHAKTHI DEVE A)

Table of Contents

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

OD	Optical Density
µg	Microgram
µl	Microlitre
µM	Micromolar
mg	Milligram
ml	Millilitre
mM	Millimolar
nm	Nanometer
cm	Centimeter
g	Gram
hrs	Hours
UV	Ultra Violet
%	Percentage
mins	Minutes
s	Seconds
α	Alpha
β	Beta
TLC	Thin Layer Chromatography
PTLC	Preparative Thin Layer Chromatography
RSM	Response Surface Methodology
LCMS	Liquid Chromatography Mass Spectroscopy
ANOVA	Analysis of Variance

CHAPTER 1

1.1 INTRODUCTION

1.1.1 DIABETES MELLITUS

Diabetes mellitus (or diabetes) is a chronic, lifelong condition that affects the body's ability to use energy found in food. There are three major types of diabetes: type 1 diabetes, type 2 diabetes, and gestational diabetes. Normally, body utilizes glucose through glycolysis and TCA cycle and converts them into ATP. In this conversion, cells need insulin, a polypeptide hormone, in the bloodstream in order to uptake glucose. With diabetes mellitus, either the body doesn't make enough insulin, it can't use the insulin it does produce, or a combination of both. High levels of blood glucose can damage the tiny blood vessels in kidneys, heart, eyes, or nervous system (www.webmd.com)

1.1.2 TYPES OF DIABETES

1.1.2.1 Type 1 diabetes (IDDM) in which the pancreas fails to secrete the insulin which is essential for the uptake of glucose in cells. This form develops most frequently in children and adolescents.

1.1.2.2 Type 2 diabetes (NIDDM) which results from the body's inability to respond properly to the action of insulin produced by the pancreas. Type 2 diabetes is much more common and accounts for around 90% of all diabetes cases worldwide. It occurs most frequently in adults, but is being noted increasingly in adolescents as well (WHO, 2002)

1.1.2.3 Gestational diabetes occurs in women, who never had diabetes before but have elevated level of blood glucose during pregnancy. Gestational diabetes affects about 4% of all pregnant women. It may precede in the development of type 2 (or rarely type 1) diabetes.

1.1.2.4 Maturity-onset diabetes of the young (MODY) includes several forms of diabetes with monogenetic defects of beta-cell function (impaired insulin secretion), usually manifesting as mild hyperglycaemia at a young age, and usually inherited in an autosomal-dominant manner (www.patient.co.uk)

1.1.3 STATISTICAL ANALYSIS OF *DIABETES MELLITUS*

Diabetes has become a common global health problem that affects >170 million people worldwide. It is one of the leading causes of death and disability. It is estimated that by 2030, the number of people with diabetes will reach 366 million (Singh, 2011). In India this increase is estimated to be 58%, from 51 million people in 2010 to 87 million in 2030 (Gupta, 2010)

In terms of rank of countries for T2DM prevalence, Ukraine (3.2 million) is at the bottom of the list, Pakistan (5.2 million) comes at number six, China is second with 20.8 million people and India has the highest number (31.7 million) of people with rate of 3% for T2DM (see Table 1.3).

Table:1.1.3 Top Ten countries for number of persons with Diabetes (Gupta., 2010)

Rank	Country	People with T2DM (million) in year 2010	Rank	Country	People with T2DM (million) in year 2030
1	India	31.7	1	India	79.4
2	China	20.8	2	China	42.3
3	USA	17.7	3	USA	30.3
4	Indonesia	8.4	4	Indonesia	21.3
5	Japan	6.8	5	Pakistan	13.9
6	Pakistan	5.2	6	Brazil	11.3
7	Russia Fed	4.6	7	Bangladesh	11.1
8	Brazil	4.6	8	Japan	18.9
9	Italy	4.3	9	Philippines	7.8
10	Ukraine	3.2	10	Egypt	6.7

1.1.4 COMPLICATIONS ASSOCIATED WITH DIABETES MELLITUS

Diabetic retinopathy is a leading cause of blindness and visual disability. Diabetes mellitus is associated with damage to the small blood vessels in the retina, resulting in loss of

vision. Loss of vision due to certain types of glaucoma and cataract may also be more common in people with diabetes than in those without the disease.

Diabetes is among the leading causes of **Diabetic nephropathy**, but its frequency varies between populations and is also related to the severity and duration of the disease. Several measures to slow down the progress of renal damage have been identified. They include control of high blood glucose, control of high blood pressure, intervention with medication in the early stage of kidney damage, and restriction of dietary protein.

Cardiovascular disease accounts for approximately 50% of all deaths among people with diabetes in industrialized countries. Risk factors for heart disease in people with diabetes include smoking, high blood pressure, high serum cholesterol and obesity.

Diabetic neuropathy is probably the most common complication of diabetes. Studies suggest that up to 50% of people with diabetes are affected to some degree. Major risk factors of this condition are the level and duration of elevated blood glucose. Neuropathy can lead to sensory loss and damage to the limbs. It is also a major cause of impotence in diabetic men. Diabetic foot disease, due to changes in blood vessels and nerves, often leads to ulceration and subsequent limb amputation. It is one of the most costly complications of diabetes, especially in communities with inadequate footwear. It results from both vascular and neurological disease processes. Diabetes is the most common cause of non-traumatic amputation of the lower limb, which may be prevented by regular inspection and good care of the foot (www.who.in)

1.1.5 GLYCATION PROCESS

Non-enzymatic protein glycation, oxidative stress and lipid peroxidation are the fundamental processes which are closely associated with various biological disorders particularly diabetes and late diabetic complications (Abbas *et al.,* 2012). Protein glycation occurs between blood sugar and protein moities such as collagen and hemoglobin, to form AGEs, which leads to the degradation of the protein. Oxidative reaction is known to be included in glycation process. Combined glycation and oxidation, "glycoxidation" occurs when Amadori compounds are degraded to form even more reactive dicarbonyl compounds (Lee *et al.,* 2011). The glycoxidation products N^{ϵ}-(carboxymethyl) lysine and pentosidine are observed to be increased in skin collagen with age and severing in diabetes (Knecht *et al*.,1997).

Advance glycation end-products (AGEs) are generated in the diabetic mellitus as a result of chronic hyperglycemia and enhanced oxidative stress. Therefore, agents with antiglycation and antioxidant properties may retard the process of AGEs formation by preventing further oxidation of metal-catalyzed glucose oxidation (Mukherjee *et al*., 2012). Oxidative reaction is known to be included in glycation process. Combined glycation and oxidation, "glycoxidation" occurs when Amadori compounds are degraded to form even more reactive dicarbonyl compounds. Carboxyl-methllysine (CML) and carboxymethyl-hydroxylysine (CMhL) are AGEs formed by oxidative cleavage of Amadori adducts, whereas pentosidine is formed between crosslinking of lysine and arginine. The formation of CML and CMhL from Amadori compounds can be inhibited by free radical scavengers, and formation of pentosidine can be inhibited under anaerobic condition

Theories involving free radicals and glycation processes are commonly used to explain the mechanism of skin aging. Aging proceeds by means of highly complicated biochemical processes in which the involvement of reactive oxygen species (ROS) and free radicals have been implicated. The overproduction of ROS and reactive nitrogen species (RNS) is a common underlying mechanism of aging, as they can damage various cellular components, including proteins, lipids and DNA (Povichit *et al.,* 2010)

1.1.6 OVERVIEW OF MEDICINAL PLANTS IN DIABETES

Advanced glycation end-products (AGEs) and oxidative stress have been implicated in the pathogenesis of diabetic complications. Both are known to interact with each other. Therefore, natural compounds or extracts that possess both antioxidant and antiglycation activities might have great therapeutic potential for treating diabetic complications (Chen *et al*., 2011).

Extracts of ***Asteracantha longifolia*** **Nees.** can significantly improve glucose tolerance in healthy human subjects and diabetic patients. Seeds of ***Coriandrum sativum*** **L**(Coriander)., reduce the hyperglycaemia during the development of streptozotocin-induced diabetes in mice. The antidiabetic activity of ***Mangifera indica*** **L**(Mango) helps in reduction of glucose absorption during hypoglycaemia (M Bnouham *et al*., 2006)

In Chinese Traditional Medicine, the style of ***Zea mays*** (corn silk) is used as a diuretic and for the prevention of diabetic complications. Corn silk contains phenolic compounds of which flavonoids, such as luteolin and maysin, are abundant (Arthur Farsi *et al.,* 2007)

Antioxidant and glycationinhibitory activity of solvent extract from different portions of gold kiwifruit (A. chinensis), and their flavonoid and polyphenol contents were investigated by Lee *et al.,* 2011. It proved that effect of A. chinensis rind ethyl acetate fraction on the inhibition of AGE formation in a glycated collagen model.

Syzygium cumini (Jambul) belongs to Myrtaceae family. The barks, leaves and seed extracts of Jambul have reported to possess anti-inflamatory, anti-bacterial and anti-diarrheal effect (Kumar *et al*., 2008). **Grape seed procyanidins** (GSPCs) are a family of bioflavonoid polymers, have a variety of health benefits due to their antibacterial, antiviral, anticarcinogenic, anti-inflammatory, and vasodilatory actions (Chuan *et al.,* 2009). *Vitis vinifera* is known to have anti-oxidant and vasodilative properties.

Citrus extract containing high levels of flavanoneglycosides could beused to control the blood glucose level of diabetic patients by inhibiting R amylase and R glucosidase in the intestinal tract. Further studies suggest that Citrus flavanones suggest that naringenin is able to reduce glucose uptake and inhibit intestinal and renal Na^{+} -glucose co-transporter (Ramful *et al*., 2010). Studies have explored the anti-inflammatory role of ***Artocarpus heterophyllus*** (jackfruit), which can be important for the prevention of the progression of obesity-associated low grade inflammation and its complications (Devalaraja et al., 2011). Seeds of ***Mannilkara zapota*** are antipyretic, and when ground with water they act as a diuretic. Antimicrobial and antioxidant activities are also reported from the leaves of *Manilkara zapota* (Sapodilla) (Chanda *et al*., 2010)

Table 1.1.6 Overview of medicinal plants

S.No	Name of the plant	Parts used	Uses	References
1.	Citrus sinensis (Orange)	Pulp	Anti-oxidant	Ramful *et al*., 2010
2.	*Coriandrum sativum* L (Corriander leaves)	Seeds	Reduction of hyperglycaemia	Bnouham *et al*., 2006
3.	*Mangifera indica* L (Mango)	Seeds, leaves	Anti-diabetic, anti-fungal	Petchi *et al.,* 2011
4.	*Syzygium cumini* (Jambul)	Barks, leaves, seeds	Anti-bacterial, anti-diabetic	Kumar *et al*., 2008
5.	*Vitis vinifera (Grapes)*	seeds	anti-oxidant and vasodilative properties	Chuan *et al.,* 2009
6.	*Mannilkara zapota* (Sapodilla)	Seeds	*Antimicrobial, anti-oxidant*	Chanda *et al*., 2010
7.	*Artocarpus heterophyllus* (jackfruit)	Fruits	Prevention of the progression of obesity-associated low grade inflammation	Devalaraja *et al.,* 2011

1.1.7 POLYPHENOLS

Phenolic compounds are secondary plant metabolites and are involved in a wide range of specialized physiological functions. They are very important for the normal growth, development and defense mechanisms of plants. These compounds are capable of inhibiting free radicals, and hence can retard the aging process (Povichit *et al.*, 2010).

Dietary polyphenols represent a wide variety of compounds that occur in fruits, vegetables, wine, tea, extra virgin olive oil, chocolate and other cocoa products. They are mostly derivatives and/or isomers of flavones, isoflavones, flavonols, catechins and phenolic acids, and possess diverse biological properties such as antioxidant, anti-apoptosis, anti-aging, anticarcinogen, anti-inflammation, anti-atherosclerosis, cardiovascular protection, improvement of the endothelial function, as well as inhibition of angiogenesis and cell proliferation activity (Han *et al.,* 2007).

Polyphenols, rather than exerting direct antioxidant effects, the mechanisms by which polyphenols express these beneficial properties appear to involve their interaction with cellular signaling pathways and related machinery that mediate cell function under both normal and pathological conditions (Vauzour *et al*., 2010).

Flavonoids are a group of about 4000 naturally occuring polyphenolic compounds, found in fruits, vegetables, nuts, seeds, spices, stems, flowers, as well as in tea and red wine. They are usually subdivided according to their substituents into flavonols (kaempferol, quercetin), anthocyanins, flavonons, flavons, chalcones. These flavonoids display a remarkable array of biochemical pharmacological actios via. anti-inflammatory, anti-oxidant, anti-allergic, hepatoprotective, anti-thrombotic, anti-viral and anti-carcenogenic activities (Meena *et al.,* 2008).

1.1.8 OBJECTIVES

- To investigate the *in-vitro* antiglycation activity for the selected fruit seeds
- To extract the polyphenolic compounds from seed extracts using different extraction methods (Hot water extraction, Pressurized hot water extraction and Solvent extraction)
- To optimize the extraction conditions for antiglycation glycation activity (%) using RSM
- To identify and isolate the polyphenolic content using TLC and PTLC and predict the polyphenolic compounds using LCMS

1.2 REVIEW OF LITERATURE

1.2.1 DIABETES

Diabetes is associated with long-term complications that affects almost every organ of the body. The disease often leads to blindness, heart and blood vessel disease, stroke, kidney failures, amputations and nerve damage. Uncontrolled diabetes can complicate pregnancy and birth defects are more common in babies born to women with diabetes. The majority of diabetes (~90%) is type 2 diabetes (T2D) caused by a combination of impaired insulin secretion from pancreatic beta cells and insulin resistance of the peripheral target tissues, especially muscle and liver (Singh, 2011).

1.2.2 PATHOPHYSIOLOGY

Type 2 diabetes is characterized by decreased disposal of glucose in peripheral tissues due to insulin resistance and Overproduction of glucose by the liver, defects in pancreatic *β*-cell function, and decreased *β*-cell mass. Obesity, decreased physical exercise, and consumption of foods with a high glycemic index (GI) and load are major predisposing factors in the development of type 2 diabetes. The GI is used to evaluate the rise in blood glucose levels in response to food. The GI provides an indication of the quality of carbohydrate in a food. The glycemic load (GL) is used to provide information about the quantity of carbohydrates in a food and the insulin demand (Susan, 2009).

1.2.3 MECHANISM OF ACTION

Enzymes involved in metabolism can be either activated or inactivated by phosphorylation. The protein kinases that catalyze phosphorylation of glycogen phosphorylase and hormone-sensitive lipase are controlled through cyclic nucleotides (PKA and cyclic AMP), Ca++ and diacylglycerol (PKC) and PI(3,4,5P)P3 (PKB). The extent of enzyme phosphorylation is controlled by the balance between protein kinases and protein phosphatases. Hormone-sensitive lipase activity in fat cells is regulated largely through cAMP activation of protein kinase A (PKA). The cyclic nucleotide levels is controlled through the balance between hormone-regulated G-protein control of adenylate cyclase and breakdown of cAMP catalyzed by phosphodiesterase. Insulin regulates cAMP levels through its stimulatory effect on the esterase and reduction of cAMP levels (www.medbio.info)

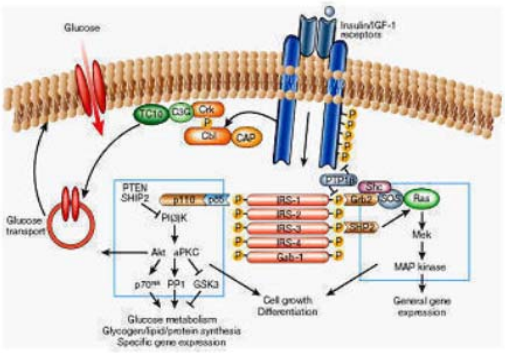


Fig 1.2.3 Mechanism of action of insulin

1.2.4 INSULIN PRODUCTION AND FAILURE OF SECRETION

Glucose uptake to muscle and fat cells is dependent upon activation of GLUT4 by insulin. This system fails when insulin secretion or when the body's responsiveness is no longer coupled to blood glucose levels. The liver's gluconeogenesis progresses without alteration following reduction or loss of the insulin signal, releasing sugar in spite of high blood glucose levels. The body reacts as though glucose was not present. Lipolysis and hepatic gluconeogenesis are activated by glucagon, growth hormone and catecholamines to meet this "low energy crisis". Massive amounts of fatty acids are released to the circulation and the liver converts these to ketone bodies. The high blood glucose levels lead to diuresis with loss of water, Na+, K+ and glucose, while the "ketones" (which are actually carboxy acids) lead to a pronounced fall in blood pH. Diabetic coma and death follow if effective treatment is not initiated (www.medbio.info)

1.2.5 ANTIGLYCATION

Non-enzymatic protein glycation (Amadori product), oxidative stress and lipid peroxidation are the fundamental processes which are closely associated with various biological disorders particularly diabetes and late diabetic complications (Abbas *et al.*, 2012). The oxidation process is believed to play an important role in AGEs formation. Further oxidation of Amadori product leads to the formation of intermediate carbonyl compounds that can react with the nearby lysine or arginine residues to form protein crosslink and AGEs. The reactive carbonyl compounds may also be generated from the metal ion-catalyzed auto oxidation of glucose. Therefore, agents with anti oxidative or metal-chelating property may retard the process of AGEs formation by preventing further oxidation of Amadori product and metal-catalyzed glucose oxidation. In this regard, several natural compounds known to possess anti oxidative property, such as curcumin, rutin, garcinol and flavonoid-rich extracts, have been shown to prevent AGEs formation *in vitro* and *in vivo* (Jedsadayanmata, 2005).

1.2.6 OXIDATIVE STRESS

Oxidative stress results in oxidative alteration of biological macromolecules such as lipids, proteins and nucleic acids. It is considered to play a pivotal role in the pathogenesis of aging and degenerative diseases. In order to cope with an excess of free radicals produced upon oxidative stress, system have developed sophisticated mechanisms for maintaining redox homeostasis. These protective mechanisms include scavenging or detoxification of reactive oxygen species (ROS), blocking ROS production, sequestration of transition metals, as well as enzymatic and non enzymatic antioxidant defenses produced in the body, that is, endogenous, and others supplied with the diet, namely, exogenous ones (Han *et al*., 2007). Oxidative reaction is known to be included in glycation process. Combined glycation and oxidation, "glycoxidation" occurs when Amadori compounds are degraded to form even more reactive dicarbonyl compounds. Carboxyl-methllysine (CML) and carboxymethyl-hydroxylysine (CMhL) are AGEs formed by oxidative cleavage of Amadori adducts, whereas pentosidine is formed between crosslinking of lysine and arginine. The formation of CML and CMhL from Amadori compounds can be inhibited by free radical scavengers, and formation of pentosidine can be inhibited under anaerobic condition (Lee *et al.*,2011).

1.2.7 FRUITS SELECTED FOR INVESTIGATION

1.2.7.1 *Vitis vinifera* (Grapes)

Grape seed procyanidins (GSPCs) are a family of bioflavonoid polymers that were once known as "vitamin P". GSPCs have unique chemical structures comprising dimers or trimers of catechin and (-)-epicatechin (EC) and are found in red wine and grape seeds. A number of studies have indicated that they have a variety of health benefits due to their antibacterial, antiviral, anti carcinogenic, anti-inflammatory, and vasodilatory actions. Further, procyanidins restore post ischemic function in isolated rat hearts, stimulate the activities of enzymes, such as tyrosine kinases and phosphoinositide kinases [13-14], may prevent human metabolic syndrome, and possess anti oxidantand free radical-scavenging activities (Chuan *et al.,* 2009)

Fig 1.2.7.1 Seeds of *Vitis vinifera*

1.2.7.2 *Mangifera indica* (Mango)

The investigation of anti-diabetic agents of plant origin which are used in traditional medicine is that of great importance. The leaf of *Mangifera indica* has been used in the indigenous systems of medicine in India for the treatment of several diseases (Basha *et al.,* 2011). The ethanolic extract of kernel seeds anf leaves of *Mangifera indica* showed significant hypoglycaemic effect in alloxan induced diabetic rats (Petchi *et al.,* 2011)

The seed kernel of *Mangifera indica* is one such herbal source which is mentioned in Ayurvedic literature for treating Diabetes mellitus. The kernel is astringent, anti-helmintic, stimulant, anti-inflammatory, antibacterial, antifungal, anti-pasmodic, anti-scorbutic and is administered in asthma, diabetes, nasal bleeding, diarrhea and ulcers (Naveen jain *et al.,* 2011).

Fig 1.2.7.2 Seeds of *Mangifera indica*

1.2.7.3 *Syzygium cumini* (Jambul)

Syzygium cumini (Myrtaceae) is widely used traditional system of medicine to treat diabetes in India. The study was conducted to isolate and identify the putative anti-diabetic compound from *S.cumini* seeds. A compound mycaminose was isolated from SC seed extracts. This compound produced significant reduction in blood glucose level (Kumar *et al.,* 2008)

Fig 1.2.7.3 Seeds of *Syzygium cumini*

1.2.7.4 *Artocarpus heterophyllus* (Jackfruit)

The ethanol extracts of *Artocarpus heterophyllus* in diabetic rats at a dose level of 400 mg/kg significantly reduced the blood glucose level, which revealed that the *Artocarpus heterophyllus* extract has significant anti-hyperglycaemic activity (Patel *et al.,* 2012)

Fig 1.2.7.4 Seeds of *Artocarpus heterophyllus*

1.2.7.5 *Citrus sinensis* (Orange)

The natural antioxidants are effective on adipocyte response to oxidative stress. Using a diabetes-like oxidative stress model, the potential protective effect of anti oxidative and pulp extracts of Citrus sinensis and C. reticulata was investigated by Ramful et al., 2010 on human adipocytes. Besides the retardation of free-radical-induced hemolysis of human erythrocytes, non-cytotoxic concentrations of extracts significantly reduced the levels of protein carbonyls in response to advanced glycation end products (AGEs) generated by albumin glycation in SW872 cells. C.sinensis extracts lowered carbonyl accumulation in H2O2-treated adipocytes

Fig 1.2.7.5 Seeds of *Citrus sinensis*

1.2.7.6 *Manilkara zapota* (Sapota)

The antioxidant capacity of the *Manilkara zapota* L. leaves extracts, obtained by sequential extraction with different polarities of solvents, was evaluated by four different *in vitro* methods: DPPH, superoxide and hydroxyl radical scavenging activity and reducing capacity assessment assay. The antioxidant capacity of acetone extract may be due to its high phenolic content. The high antioxidant capacity observed for acetone extract suggested that this plant could be used as an additive in the food industry providing good protection against oxidative damage (Chanda *et al.,* 2010)

Fig 1.2.7.6 Seeds of *Manilkara zapota*

1.2.8 RESPONSE SURFACE METHODOLOGY (RSM)

RSM comprises a body of methods for exploring for optimum operating conditions for experimental methods. This involves doing several experiments, using the result of one experiment to provide direction to focus on experiments with different set of conditions, or to collect more data in present experimental region in order to fit higher-order model or confirm which was found. The RSM package for R provides several functions to facilitate classical response-surface methods as described by Box and Draper, Khuri and Cornell, Wu and Hamada, Myers and Montgomery, Box, Hunter and Hunter and Ryan (Lenth., 2009).

1.2.9 EXTRACTION PROCESS

Efficiency of solvents and methods are strongly dependent on plant matrix used. Solvents, such as methanol, ethanol, acetone, propanol and ethyl acetate have been commonly used for the extraction of phenolics from fresh product. The properties of extracting solvents significantly affect the measured total phenolics content and antioxidant capacity in fruits and vegetables (Tomsone *et al.,* 2012). For extraction of polyphenols from plant matter different solvent systems should be used depending on their efficiency. Extraction yield depends on the solvent and method of extraction. Commonly used solvents for extracting various substances from plant material are water, aqueous mixtures of ethanol, methanol and acetone (Jakopic *et al*., 2009). Accelerated Solvent Extraction (ASE) is an automated extraction technique that rapidly performs solvent extractions using high temperatures and pressure. The automation of ASE allows for high sample throughput and, if needed, fast method development. It produces solvent extracts with similar or better recoveries than traditional extraction techniques (www.thermo scientific.com)

The extraction procedure is sequential and systematic, carried out using an aqueous organic solvent to extract phenolic compounds in fruit and vegetable samples. This traditional method is called liquid-liquid extraction (LLE) and different extraction solvents such as ethanol, acetone or methanol, or a mixture with water. Soxhlet system is used to extract the lipidic fraction from food and other solid samples, using suitable solvents. Although it is not specific for phenolic compounds extraction, usually the extraction yields are compared to those obtained with another type of polyphenol extraction systems. Modern extraction and isolation techniques can be used as alternative techniques to considerably reduce solvent consumption and accelerate the extraction process. These modern techniques include: supercritical fluid extraction (SFE), pressurized liquid extraction (PLE), microwave-assisted extraction (MAE) and ultrasound-assisted extraction (UAE) (Garcia *et al*., 2010)

1.2.10 POLYPHENOLS

Polyphenols represent a group of secondary metabolites which widely occur in fruits, vegetables, wine, tea, extra virgin olive oil, chocolate and other cocoa products. They are mostly derivatives, and/or isomers of flavones, isoflavones, flavonols, catechins, and phenolic acids. Phenolic acids account for about one third of the total intake and flavonoids account for the remaining two thirds. Dietary polyphenols exhibit many biologically significant functions, such as protection against oxidative stress, and degenerative diseases. Dietary polyphenols may offer an indirect protection by activating endogenous defense systems and by modulating cellular signaling processes such as NF-κB activation, AP-1 DNA binding, glutathione biosynthesis, PI3-kinase/Akt pathway, MAPK proteins (ERK, JNK and P38) activation, and the translocation into the nucleus of Nrf2 (Han *et al*., 2007)

1.2.10.1 Phenolic acids

A major class within the phenolic compounds is the hydroxycinnamic acids, which are widely distributed in plant kingdom. The major hydroxycinnamic acid is caffeic acid, which occurs in foods mainly as an ester with quinic acid called chlorogenic acid (5-caffeoylquinic acid). Chlorogenic acid and caffeic acid are antioxidants *in vitro* and they might inhibit the formation of mutagenic and carcinogenic *N*-nitroso compounds for the inhibitory effect on the *N*-nitrosation reaction *in vitro* (Han *et al.,* 2007).

1.2.10.2 Flavonoids

Flavonoids constitute one of the most characteristic class of compounds in higher plants. They are water soluble pigments which occur almost universally in higher plants. They impart color to flowers, fruits and a correlation between flower color and attraction of pollinating insects is very well known. They also provide resistance to plants (Upman *et al.,* 2011).

Naturally occurring flavonoids are generally classified into six classes according to their chemical structures i.e. flavanones, flavones, isoflavonoids, flavans (flavanols), flavonols and anthocyanins by the structure of the C-ring and the functional groups at C-3 and C-4. (Li *et al*., 2007). Quercetin, a flavonol occurring in fruits and vegetables is a food component with proven beneficial impact on health. Its biochemical activity is well documented. It s one of the most potent antioxidants among polyphenols. Quercetin has also been demonstrated to display the antiviral, antibacterial, anti-carcinogenic and anti-inflammatory effects. In food, quercetin occurs mainly in a bound form, with sugars, phenolic acids, alcohols, etc. After ingestion, derivatives of quercetin are hydrolyzed mostly in the gastrointestinal tract and then absorbed and metabolized (Materska., 2008).

CHAPTER 2

MATERIALS AND METHODS

2.1 COLLECTION OF PLANT MATERIALS

Six different fruits that are commonly consumed are selected for screening the *in-vitro* antiglycation activity. The following fruits were purchased from the local market and the seeds were subjected for the experimental analysis.

- *Mangifera indica* (Mango)
- l*Syzygium cumini* (Jambu)
- *Vitis vinifera* (Grapes)
- *Citrus sinensis* (Orange)
- *Artocarpus heterophyllus* (Jackfruit)
- *Manilkara zapota* (Sapodilla)

2.2 PREPARATION OF THE EXTRACT

The seeds of fruits were washed with distilled water until the dust and other particles were removed the seeds were air dried and kept in hot air oven at 50ºC for overnight. All the samples were then ground into fine powder using mixer grinder. The samples were stored at 4ºC for further use.

2.2.1 Preparation of hot water extract (HWE)

In a clean dry 250 ml conical flask, 0.5 g of powdered material was weighed and extracted with 25 ml of distilled water by placing it in a boiling water bath at temperature 90ºC for 5 minutes. The suspension was filtered using Whatman No.2 filter paper, the filtrate obtained is precipitated by adding 10% of ammonium sulphate and centrifuged at 5000 rpm for 10 minutes and the supernatant is used for experimental analysis.

2.2.2Preparation of Pressurized hot water extract (PHWE)

In a clean dry pressurized vessel 0.5g of the powdered seeds was weighed and extracted with 5ml of distilled water. The vessel is placed in oil bath at 180º C, 10.027 bar pressure for 10 minutes. The resultant was used for antiglycation assay.

2.2.3Preparation of organic solvent extract

In a clean dry 250 ml of separating funnel, 5 g of powdered material was weighed and mixed thoroughly with 20 ml of petroleum ether for 15-20 mins. The filtrate was collected and the residue was reextracted with 20 ml of chloroform. The filtrate was collected and the residue was reextracted with 20 ml of acetone. The filtrate was collected and the residue was reextracted with 20 ml of ethyl acetate. The filtrate was collected and the residue was reextracted with 20 ml of ethanol. The filtrate was collected and the residue was reextracted with 20 ml of distilled water. All the samples were pooled to 1:1 ratio and the samples was used as pooled mixture.

2.3 ANTIGLYCATION ASSAY

A method provided by Majumdar *et al.,* 2012 was adopted for *in-vitro* antiglycation activity.

Reagents required

See appendix 1

Procedure

In a test tube, 10 ml of BSA and 5 ml of 500mM glucose was added. Then, 2-3 ml of Sodium azide was added along with 5 ml of samples, extracted by HWE, PHWE and organic solvent extraction and incubated at 32ºC for 5 days. About 0.5 ml of reaction mixture was pipetted in a new test tube and 2ml of 0.3mM NBT was added. The content was incubated at room temperature for 15 minutes and the absorbance was read at 530 nm spectrophotometer.

2.4 THIN LAYER CHROMATOGRAPHY (TLC) AND PREPARATIVE THIN LAYER CHROMATOGRAPHY (PTLC)

Reagents Required

See appendix 1

Procedure

Glass plates (20× 10 cm) were taken. Silica gel dissolved in water was applied as a thin layer to the glass plate with the help of an applicator. The plates were dried for 24 hrs to remove moisture or water and other adsorbed substances from the surface so as to activate the plate. 40µlof organic solvent extract of Jambul seed samples was spotted on each plate using micropipette. Spot was placed 2cm above the base of the plate and the spotting area should not be immersed in the mobile phase. The development tank has to be saturated for 24 hrs with mobile phase Ethyl acetate: Formic acid: Acetic acid: distilled water in the ration of 25: 2.75: 2.75: 6.5. The glass plates with the spotted area were immersed in the separate tanks and developed. The plates were dried before spraying liquid ammonia (detecting agent). Moderate amount of the reagent has to be sprayed to the plate so that it always appears dull and flat. The plates were then viewed under short and long UV to detect the presence of polyphenols.

The same procedure was followed for Preparative Thin Layer Chromatography (PTLC) with glass plates of size 20 cm × 20 cm and the samples were spotted along the length of the plate. The development procedure was the same as that of TLC.

The silica gel containing the flourescent band was scraped under UV light and the silica gel was centrifuged with ultra-pure distilled water. The supernatant was collected and stored at 4ºC for further use.

2.5 LC-PDA-MS (ESI+) ANALYSIS

The liquid chromatography electrospray mass spectrometry experiment was performed on Varian Inc, (USA) 410 Prostar Binary LC with 500 MS IT PDA detectors. The column was C_{18}, 250 × 4.6 mm, i.d 5µm. The mobile phase A was made up of acetonitrile while B was made of 0.1% formic acid (pH 4.0, adjusted with ammonium hydroxide) aqueous solution. The gradient elution was performed at 1ml/min with an initial condition of 12% of mobile phase A and 88% of mobile phase B for 10 mins. The mobile phase A was increased to 15% at 20 mins and linearly increased to 60% at 90 mins and then increased to 100% at 95 mins. The eluates were monitored by PDA (Multi wavelength) detector at 260nm. About 20µl of the PTLC *Artocarpus heterophyllus* (Jackfruit) seed isolates were introduced into the ESI source and the mass spectra were scanned in the range 100-1000amu and the maximum ion injection time was set at 200nS. Ion spray voltage was set at 5.3 KV and capillary voltage 34V. The MS scan ran upto 26.67 mins.

The instrument had the following specifications:

1. Direct Infusion Mass with ESI and APCI negative and positive mode ionization, mass ranging from 100 to 1000 amu.
2. LCMS ION TRAP
3. HPLC with PDA detector
4. HPLC PDA detector- Mass- Spectrometer
5. The molecular masses of phenolic acids were assigned by electrospray ionization mass.

2.6 OPTIMISATION USING RESPONSE SURFACE METHODOLOGY

To optimize the extraction conditions for flavonoids and phenolic acids, three variables were selected and they were Hexane to Heptane ratio, Time and Solid to Liquid ratio (see Table 3.7.1). The optimum extraction conditions were determined by Response Surface Methodology. A single factor analysis of variance was adopted to investigate the effect of each factor on the extraction of flavonoids and phenolic acids.

2.6.1 Experimental Design

One of the common experimental designs used for engineering purposes is a Central Composite design (CCD) that includes three variables and three factorial levels (20). The independent variables used in this study were hexane-heptane ratio, extraction time and solid-liquid ratio. Coded and uncoded levels of the independent variables and the experimental design are given in Table 2.6.1. Coded value 0 stands for centre point of the variables and is repeated for experimental error. Factorial points are coded as ±1.

Table 2.6.1. Coded and uncoded levels of independent variables used in the response surface methodology

Independent variables	Symbol	Levels		
		Low (-1)	Middle (0)	High (+1)
Hex: Hep (v/v)	X_1	5	10	15
Time (min)	X_2	2	4	6
Solid: Liquid (g/ml)	X_3	10	15	20

Second-order polynomial equation was used to express the investigated responses (Y) as a function of the coded independent variables, where X1, X2…X3 are the independent variables affecting the responses Y, β_0, β_j (i=1, 2...k), β_{ii} (i=1, 2...k), and β_{ij} (i= 1, 2…k; j=1, 2…k) are regression coefficients for intercept, linear, quadratic, and interaction terms, respectively; k is the number of variables. Coded independent variables for our experiment are solvent concentration, temperature and liquid/solid ratio.

$$Y= \beta_0 + \Sigma^{K}_{i=1} \beta_i X_i + \Sigma^{K}_{i=1} \beta_{ii} X_i^2 + + \Sigma^{K}_{i=1;i<j} \Sigma^{K}_{j=2} \beta_{ij} X_i X_j \quad \text{.....(eq. 2.6.1.)}$$

Statistical analysis was performed using **MINITAB 15 TRIAL VERSION**. The results were statistically tested by the analysis of variance (ANOVA) at the significance level of p=0.05. Response surface plots were generated with the same software and drawn by using the function of two factors, and keeping the other constant. For a total of 20 runs, 4 runs were dummy variables.

Table 2.6.2 Design of Response Surface Methodology

Std Order	Hexane: Heptane	Time (min)	Solid: Liquid
1	1:5	2	1:10
2	1:15	2	1:10
3	1:5	6	1:10
4	1:15	6	1:10
5	1:5	2	1:20
6	1:15	2	1:20
7	1:5	6	1:20
8	1:15	6	1:20
9	1:6	4	1:15
10	1:18	4	1:15
11	1:10	0.6	1:15
12	1:10	7	1:15
13	1:10	4	1:6.6
14	1:10	4	1:23.4
15	1:10	4	1:15
16	1:10	4	1:15
17	1:10	4	1:15
18	1:10	4	1:15
19	1:10	4	1:15
20	1:10	4	1:15

CHAPTER 3

RESULTS AND DISCUSSION

3.1 *IN VITRO* ANTIGLYCATION ACTIVITY OF DIFFEERNT EXTRACTION METHODS (%)

Extraction methods used pharmaceutically involves the separation of medicinally active portions of plant tissues from the inactive/inert components by using selective solvents. During extraction, solvents diffuse into the solid plant material and solubilize compounds with similar polarity (Tiwari *et al.,* 2011)

Comparison of *in vitro* antiglycation activity of six different seeds (*Syzygium cumini, Citrus sinensis, Mangifera indica, Vitis vinifera, Manilakara zapota, Artocarpus heterophyllus*) using different extraction methods was depicted in Table 3.1

Table 3.1 Comparison of antiglycation activity among different seeds using different extraction methods:

S.No	Name of the seed	Hot Water Extraction (%)	Pressurized Hot Water Extraction (%)	Solvent Extraction (%)
1.	*Syzygium cumini*	16.07	49.24	**78.14**
2.	*Citrus sinensis*	19.80	**74.79**	**75.65**
3.	*Mangifera indica*	59.54	**84.76**	65.17
4.	*Vitis vinifera*	68.87	79.86	**83.31**
5.	*Manilakara zapota*	44.72	**81.59**	81.24
6.	*Artocarpus heterophyllus*	40.92	**87.52**	82.68

Artocarpus heterophyllus seed extract showed maximum antiglycation activity (87.52 %) while *Citrus sinensis* seed extract showed manimum antiglycation activity (74.79 %) in pressurized hot water extraction.

The leaves of *A.heterophyllus,* extracted using water, decreased the blood glucose levels significantly (Shahin *et al.,* 2012). Decrease in blood glucose level leads to anti-diabetic effect. Hence, the present results were well corroborated with the above mentioned investigation that the seeds of *A.heterophyllus* showed maximum activity against glycation.

Citrus sinensis peels decrease the activity of glucose-6-phosphatase and phosphoenol pyruvate. The anti-diabetic activity of orange peels and juice appear to be mediated through anti-peroxidation, inhibition of α-amylase enzyme activity that is mainly responsible for the conversion of complex carbohydrates to glucose, increased hepatic glycogen content, stimulation of increased insulin secretion and repair of secretory defects of pancreatic β-cells (Milind *et al.,* 2012). From the above investigation it is proved that *Citrus* fruits are capable against diabetes.

According to Santos *et al.,* (2012) solvent extraction of *Syzygium cumini* seeds using soxhlet extraction method, recorded low yields of total phenol content when compared to ultrasound-assisted extraction (UAE), agitated bed extraction (ABE) and a combination of UAE and ABE (UAE+ABE).

Among six different seeds used, four recorded higher antiglycation activity in pressurized hot water extraction while solvent extraction recorded equally. Pressurized hot water extraction (PHWE) is a solid–liquid extraction process performed at elevated temperatures about 180º C and at a pressure of about 10 bars. Extraction is carried out under pressure to maintain the solvent in its liquid state at high temperature. The solvent will below its critical condition during PHWE. Increased temperature accelerates the extraction kinetics and elevated pressure keeps the solvent in the liquid state, thus achieving safe and rapid extraction. Also, pressure allows the extraction cell to be filled faster and helps to force liquid into the solid matrix. Elevated temperatures enhance diffusivity of the solvent resulting in increased extraction kinetics. The use of non-toxic extracting solvents such as carbon dioxide and water has economic and environmental benefits. Supercritical CO2 extraction has been reported to be a valuable novel extraction technique for the extraction of nutraceuticals (Wang *et al.*, 2006)

Solvent extractions are the most commonly used procedures to prepare extracts from plant materials due to their ease of use, efficiency, and wide applicability. It is generally known that the yield of chemical extraction depends on the type of solvents with varying polarities, extraction time and temperature, sample-to-solvent ratio as well as on the chemical composition and physical characteristics of the samples. The solubility of phenolics is governed by the chemical nature of the plant sample, as well as the polarity of the solvents used (Dai *et al.,* 2012).

3.2 OPTIMIZATION OF ANTIGLYCATION ACTIVITY OF *Citrus sinensis* SEEDS BY RESPONSE SURFACE METHODOLOGY

Optimization is an essential tool for the efficient operation of different processes to yield a highly acceptable product. During optimization of extraction process, several response variables describe the quality characteristics of the obtained extracts. Some of these variables need to be maximized, while others need to be minimized (Radojkovic *et al.,* 2012).

The optimization of polyphenols for *Citrus sinensis* (Orange) has been done and optimum conditions for antiglycation inhibition was found to be at Hexane : Heptane ratio (v/v) (1:15), Time (6 minutes) and Solid : Liquid ratio (g/ml) (1:20) and maximum antiglycation activity was found to be **89.79 % (15 % increase).**

Table 3.2.1 OPTIMIZATION OF ANTIGLYCATION ACTIVITY OF *Citrus sinensis* SEEDS BY RESPONSE SURFACE METHODOLOGY

Std Order	Hexane: Heptane	Time (min)	Solid: Liquid	% Antiglycation
1	1:5	2	1:10	85.38
2	1:15	2	1:10	86.89
3	1:5	6	1:10	80.00
4	1:15	6	1:10	87.24
5	1:5	2	1:20	84.27
6	1:15	2	1:20	88.97
7	1:5	6	1:20	88.76
8	**1:15**	**6**	**1:20**	**89.79**
9	1:6	4	1:15	85.31
10	1:18	4	1:15	77.66
11	1:10	0.6	1:15	81.72
12	1:10	7	1:15	87.72
13	1:10	4	1:6.6	85.86
14	1:10	4	1:23.4	72.34
15	1:10	4	1:15	78.69
16	1:10	4	1:15	72.84
17	1:10	4	1:15	74.34
18	1:10	4	1:15	71.38
19	1:10	4	1:15	69.17
20	1:10	4	1:15	74.21

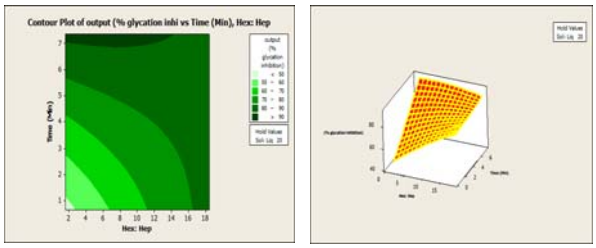
Tests were performed to select the relevant factors (Hexane : heptane ratio, time and Solid : Liquid ratio). Experimental data were fitted to a second-order polynomial model and regression coefficients obtained using **MINITAB 15 TRIAL VERSION.** The regression equation was generated based on second-order equation and was expressed as:

$$Y = 79.7176 + 3.2012\,X_1 + 3.1435\,X_2 - 2.2808\,X_3 - 0.8322\,X_1^2 + 0.4353\,X_2^2 + 0.9957\,X_3^2 - 3.6975\,X_1 * X_2 + 1.4225\,X_1 * X_3 + 4.1952\,X_2 * X_3$$

where Y represents the dependent variable (antiglycation activity) and X1, X2 and X3 represents the independent variables (hexane-heptane ratio, time and solid-liquid ratio).

3.2.2 EFFECT OF EXTRACTION CONDITIONS ON ANTIGYCATION ACTIVITY *Citrus sinunsis*

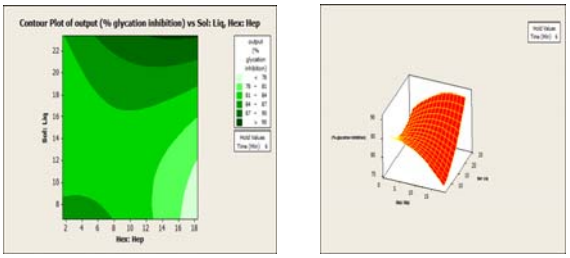
Response surface plots shows the effects of investigated parameters such as hexane-heptane ratio, time and solid-liquid ratio on their activity against glycation.



(a) (b)

Figure: 3.2.2.1 (a)Contour plot and (b) Surface plot of Output % Glycation inhibition vs. Time (min), Hexane : Heptane (v/v)

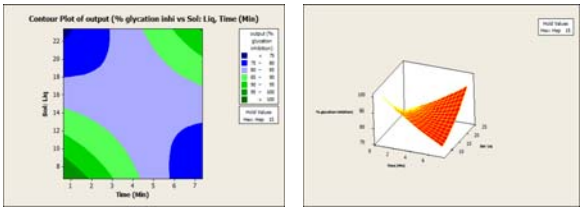
The above figure 3.2.2.1 (a) shows the contour plot of % glycation inhibition vs. time (min) and hexane: heptane (v/v) ratio. It can be predicted that at time 6minutes and subsequent increase in solvent ratio, the antiglycation activity was found to be in the range of 80-90 % and (b) shows the surface plot were there was increase in time and solvent ratio, there was higher antiglycation activity.



(a) **(b)**

Figure 3.2.2.2 (a) Contour plot and (b) Surface plot of Output % Glycation inhibition vs. Solid : Liquid (g/ml), Hexane : Heptane (v/v)

The above figure 3.2.2.2 (a) shows the contour plot and surface plot between % glycation inhibition vs. solid: liquid (g/ml) ratio and hexane: heptane (v/v) ratio. It can be predicted that at gradual increase of solid: liquid ratio and subsequent increase in solvent ratio, the antiglycation activity was found to be in the range of 81-84.



(a) **(b)**

Figure 3.2.2.3 (a) Contour plot and (b) Surface plot of Output % Glycation inhibition vs. Solid : Liquid (g/ml), Time (min)

The above figure 3.2.2.3 shows the contour plot and surface plots of % glycation inhibition vs. solid: liquid (g/ml) ratio and time (mins). It can be predicted that at gradual increase of solid: liquid ratio and subsequent increase in time, the antiglycation activity was found to be lesser in the range of 80-85 % and when there was increase in both solid: liquid ratio and time, there was decreased antiglycation activity.

3.2.3 EFFECT OF SINGLE FACTOR IN OPTIMIZATION OF *Citrus sinensis*

3.2.3.1 Effect of Hexane : Heptane ratio

The effect of Hexane : Heptane ratio on aqueous extraction was investigated over the rabge of 1:5 to 1:15 (g/ml). The extractive efficiency of phenolic compounds from plant material is greatly depended on the solvent (Jakopic *et al*., 2009).

Table 3.2.3.1 One-way ANOVA: output (% glycation inhibition) versus Hex: Hep

Source	DF	SS	MS	**F**	P
Hex: Hep	4	230.6	57.6	**1.01**	0.432
Error	15	853.5	56.9		
Total	19	1084.1			

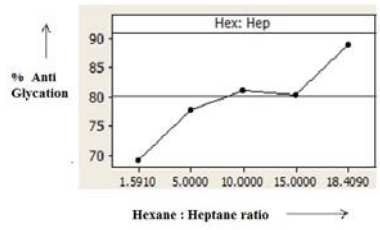


Figure 3.2.3.1 Individual effect of Hexane: Heptane ratio against antiglycation activity

The above graph depicts the action of the solvent ratio against the inhibition of glycation activity. Mahmoodally *et al*., (2012) revealed that the crude solvent extract and fragments Euphorbiaceae, Asteraceae and Lamiaceae family have potent antioxidant and antiglycation properties with no apparent cytotoxicity and might have prophylactic and therapeutic potentials in the management of diabetes and related complications. It can be predicted that when there is increase in solvent ratio, there is increase in antiglycation activity.

As *in vitro* experiments demonstrated by Davis *et al.,* (2012), hexane extract has the ability to stimulate insulin signaling events (IR-β and IRS-1 phosphorylation, PI3 kinase and GLUT4 mRNA expression) in parallel with glucose uptake, the effect of hexane extract on blood glucose, triglyceride and cholesterol has been tested in ob/ob mice. An oral administration of hexane extract for 21 days caused significant reduction in random glucose. Further, hexane extract showed a significant reduction in plasma triglyceride (30.5%).

3.2.3.2 Effect of Time (min)

The optimum time for the maximum antiglycation activity was found to be 6 minutes. The range of time was determined based on the practical and economical aspects. Increase in time from 12 to 24 h during conventional solvent extraction was found to have significant effects on the yields of total phenols and antioxidant activities from red grape. (Kashif *et al.,* 2009). The following results also shows the significant increase in antiglycation activity due to increase in time.

Table: 3.2.3.2 One-way ANOVA: output (% glycation inhibition) versus Time (Min)

Source	DF	SS	MS	**F**	P
Time (Min)	4	180.6	45.1	**0.75**	0.573
Error	15	903.5	60.2		
Total	19	1084.1			

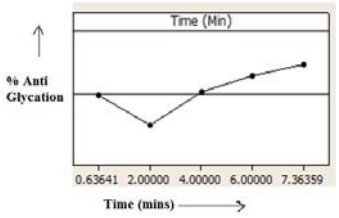


Figure 3.2.3.2 Individual effect Time against antiglycation activity

The above graph depicts the action of the time against the inhibition of glycation activity. Increase in time from 12 to 24 h during conventional solvent extraction was found to have significant effects on the yields of total phenols and antioxidant activities from red grape (Kashif *et al.,* 2009). Hence, increase in time lead to increased antiglycation activity.

3.2.3.3 Effect of Solid : Liquid (g/ml) ratio

The effect of Solid : Liquid ratio plays a major role in increasing the % antiglycation activity of *Citrus sinensis* seeds. The following results also shows the significant increase in antiglycation activity due to increase in solid and liquid ratio.

Table 3.2.3.3 One-way ANOVA: output (% glycation inhibition) versus Sol: Liq

Source	DF	SS	MS	**F**	P
Sol: Liq	4	123.2	30.8	**0.48**	0.750
Error	15	960.9	64.1		
Total	19	1084.1			

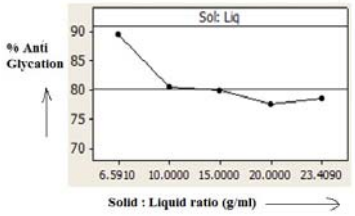


Figure 3.2.3.3 Individual effect Solid: Liquid ratio against antiglycation activity

The above graph depicts the action of the Solid: Liquid ratio against the inhibition of glycation activity. It can be predicted that, when there is increase in Solid-to-liquid ratio, there is susequent decrease in the antiglycation activity.

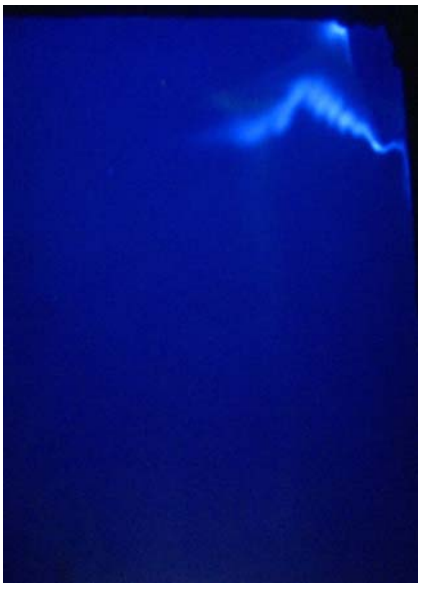
The effect of the solid to liquid ratio on the extraction yields of the four main vinca alkaloids was observed by Mu *et al.,* (2012) determined series of experiments with different solid to liquid ratios (1:10, 1:15, 1:20, 1:25 and 1:30, w/v). The extraction efficiency increased with increasing solid to liquid ratios up to 1:20. Less extraction solvent results in the insufficient mixing of sample and solvent. Higher solvent volumes did not significantly improve the extraction efficiency; the turbulent phase may be subject to restriction and destruction resulting in weaker cavitation effects.

3.3 ISOLATION AND IDENTIFICATION OF POLYPHENOLS OF *Artocarpus heterophyllus* (JACKFRUIT) SEEDS USING VARIOUS CHROMATOGRAPHIC TECHNIQUES

3.3.1 Thin Layer Chromatography (TLC)

TLC is used for the analysis of botanical raw materials. The advantages of using TLC to construct the fingerprints of herbal medicines are its simplicity, versatility, high velocity, specific sensitivity and simple sample preparation. Thus, TLC is a convenient method of determining the quality and possible adulteration of herbal products (Mohammad *et al.,* 2010).

The jackfruit seed extract revealed an R_f value of about 0.93. The UV detection of polyphenols was observed.



Identified polyphenols from two-dimensional TLC plate of Jackfruit seed extract

Figure: 3.3.1 2D Thin Layer Chromatography of PHWE of Jackfruit seed extract

A classical stationary phase of silica gel is widely used to separate many polyphenols such as phenolic acids and flavonoids. Mobile phase such as Ethyl acetate : Formic acid : Water (17 : 2 : 3) and Acetone : Acetic acid (17 : 3) has been reported by Mohammad *et al.,* 2010 for effective identification and separation of polyphenolics. The polyphenolic compounds was observed under 366nm wavelength.

3.3.2 Preparative Thin Layer Chromatography (PTLC)

PTLC has been considered as the most basic equipment for the best separation and purification of polyphenols like flavoniods and phenolic acids. It is also used for monitoring the reactions in a large scale manner and is a method that requires the least financial outlay. PTLC in conjunction with open-column chromatography used for purifying natural products. The spot observed in PTLC of Jackfruit extract was successfully isolated and subjected for LC-MS analysis.

Identified polyphenols from two-dimensional PTLC plate of Jackfruit seed extract

Figure: 3.3.2 2D Preparative Thin Layer Chromatography of PHWE of Jackfruit seed extract

The colour from the above plates shows the presence of polyphenols. Polyphenols represent a group of secondary metabolites which widely occur in fruits, vegetables, wine, tea, extra virgin olive oil, chocolate and other cocoa products. They are mostly derivatives, and/or isomers of flavones, isoflavones, flavonols, catechins, and phenolic acids. Phenolic acids account for about one third of the total intake and flavonoids account for the remaining two thirds. Dietary polyphenols exhibit many biologically significant functions, such as protection against oxidative stress, and degenerative diseases (Han *et al.,* 2007). The antiglycation activity of PTLC isolate of Jackfruit was found to be **95.38 %** .

3.3.3 Liquid Chromatography-Mass Spectroscopy (LC-MS)

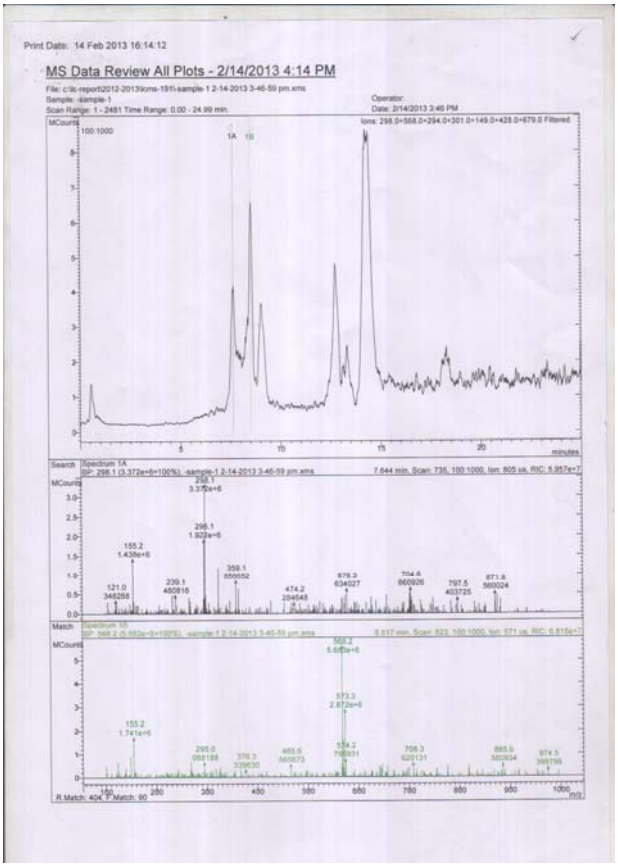
In recent years LC/MS has made a transition from a developmental stage to a more mature level where the technique is now being applied on a regular basis to many problems. Recently some of the various types of LC/MS interfaces are being used on a routine basis. The various forms of separations in the liquid phase, such as high performance liquid chromatography (HPLC), ion chromatography (IC), and capillary zone electrophoresis (CZE), are well known for their analytical power in separating complex sample (Linscheid *et al.,* 1994).

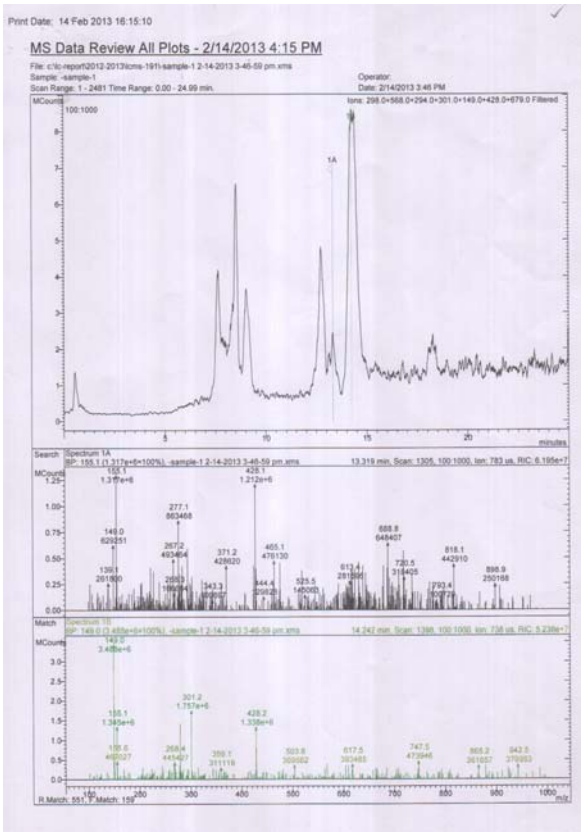
Applications of LCMS to measure the stable isotopic enriching tracers have been very limited. The primary advantage of using LCMS is that sample preparation is reduced and no derivatization need to be performed (McIntosh *et al.,* 2001). Mass spectrometer is an instrument in which the number of ions is determined electrically. There are four basic components That are standard in all mass spectrometer. These are: a sample inlet, an ionization source, a mass analyzer and an ion detector (Cronly., 2011)

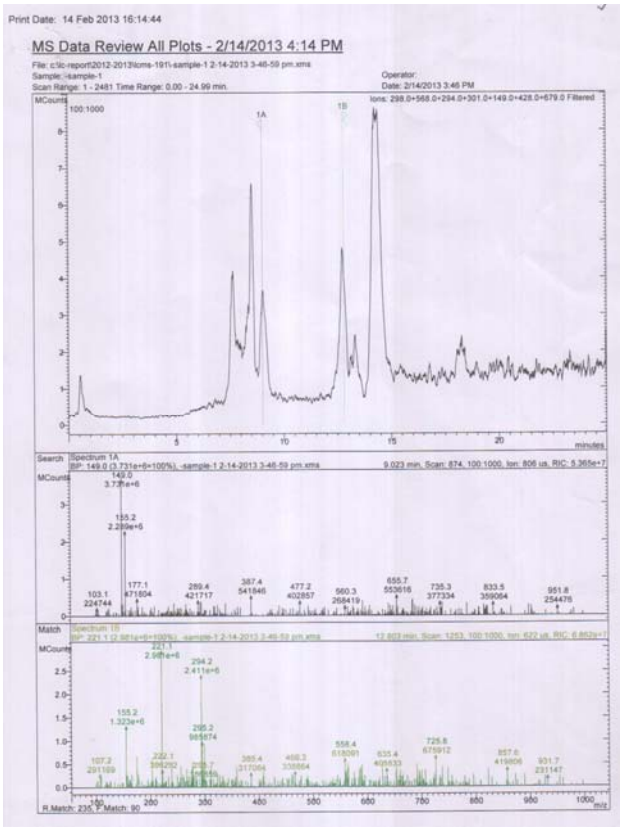
LC coupled with MS has proven to be a powerful technique for the on-line identification of target compounds in a complex mixtures, especially crude plant extracts. Moreover, the recent development of new commercial mass spectrometric systems makes possible the rapid and accurate characterization of structural features of flavonoids through the analysis of either pure compounds or of actual extracts samples (Colombo *et al.,* 2008).

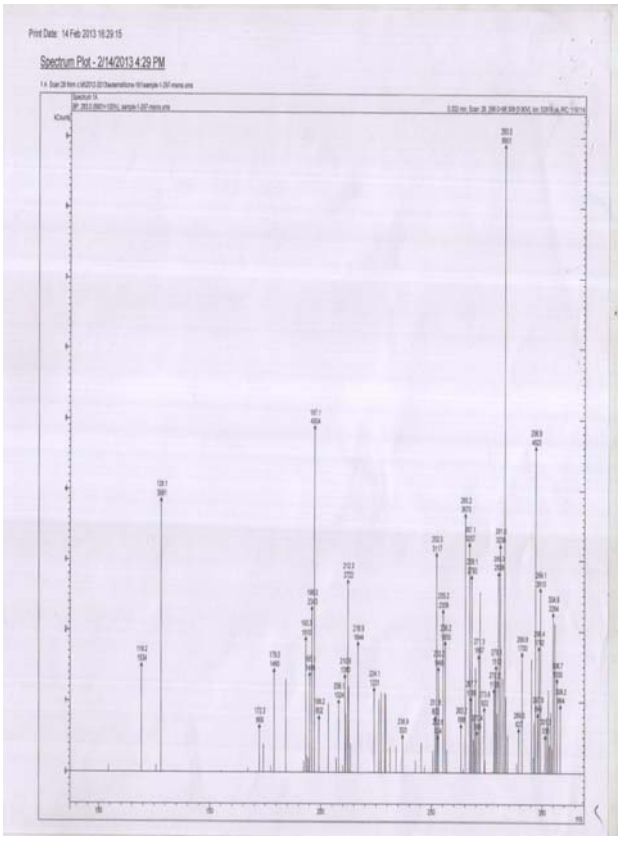
Due to abundance of different classes of flavonoids and phenolic acids and their diverse chemical properties, a variety of separation and identification methods have been developed using TLC, PTLC and LCMS.

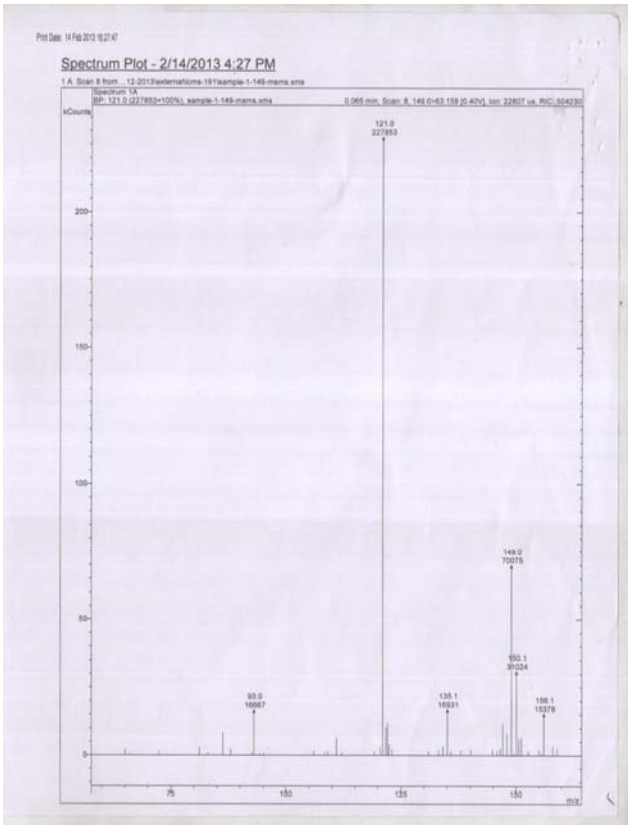
The MS data is as follows.











Fragmentation behaviour of phenolic acids and flavonoids was investigated using ion trap mass spectrometry in negative mode. The fragmentation rule in mass spectrum offers the ability to identify the related unknown compounds. The MS and UV data together with HPLC in the jackfruit extract. The compounds present are

Table: 3.4.4.1 Validation of LC-MS report

S.No	Compound	Molecular Weight	MS^1 Base peak ion	MS^2 Parent ion
1.	Rhamnosyl-dihexosyl methyl Quercetin	787	797.5	
2.	Diosmetin	299	298.1	
3.	Rutin	610.52	613.4	
4.	Quercetin-3-o-xyloside	434.86	428.2	
5.	Quercetin	301.2	301.2	
6.	4-hydroxy phenyl acetic acid	151.0	149.0	301.2
7.	4-hydroxy benzaldehyde	122.12	121.0	149.0
8.	Rhamnosyl-di hexosyl Quercetin sulphate	853.0	857.6	931.7
9.	Luteolin	286.24	283.0	299.1

According to Lin *et al.,* (2010), the MS/MS spectrum of standard Luteolin revealed two distinct peaks at m/z 287 and 285 and base peaks with m/z 256, 266. A similar MS/MS spectrum has been recorded in the present study at m/z 283.0 and base peak at m/z 299.1 has proved the presense of **Luteolin** in the sample.

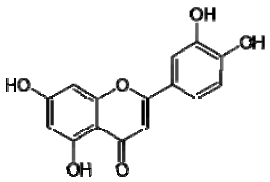


Fig: 3.4.5.1 Structure of Luteolin

According to Schiebber *et al*., (2003) the MS/MS spectrum of standard quercetin xyloside revealed the peaks at m/z 433 and base peak with m/z with 301 and 300. A similar spectrum has been recorded in the present study at m/z 428.2 with base peak at m/z 428 has proved the presence of **Quercetin-3-o-xyloside** in the sample.

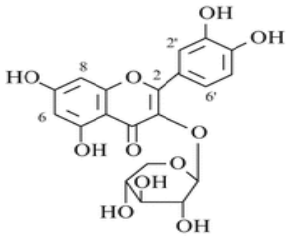


Fig: 3.4.5.2 Structure of Quercetin-3-o-xyloside

According to Lin *et al.,* (2010), the MS/MS spectrum of standard Diosmetin revealed two distinct peaks at m/z 301 and 299. A similar MS/MS spectrum has been recorded in the present study at m/z 298.1 which has proved the presense of **Diosmetin** in the sample.

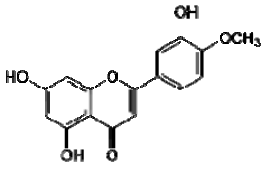


Fig: 3.4.5.3 Structure of Diosmetin

According to Justesen *et al.,* (2000), the MS/MS spectrum of standard Quercetin revealed peaks at m/z 179 and 121 with base peak at m/z 301. A similar MS/MS spectrum has been recorded in the present study at m/z 301.2 which has proved the presense of **Quercetin** in the sample.

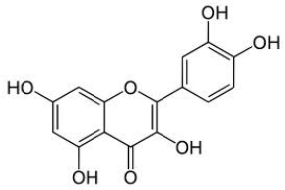


Fig: 3.4.5.6 Structure of Quercetin

According to Hong *et al.,* (2006), the MS/MS spectrum of standard Quercetin revealed distinct peaks at m/z 641, 479 and 317 with base peak at m/z 786. A similar MS/MS spectrum has been recorded in the present study at m/z 787 and base peak with m/z 797.5 which has proved the presense of **Rhamnosyl dihexosyl methyl Quercetin** in the sample.

According to Hong *et al.,* (2006), the MS/MS spectrum of standard Quercetin revealed peaks at m/z 773, 627, 465 and 303 with base peak at m/z 853. A similar MS/MS spectrum has been recorded in the present study at m/z 857.6 and base peak with m/z 931.7 which has proved the presense of **Rhamnosyl dihexosyl Quercetin sulphate** in the sample.

APPENDICES

Appendix 1

3.3 Antiglycation assay

Reagents required

- Bovine Serum Albumin (BSA 20mg/ml)
- Glucose (500mM)
- 0.02% Sodium azide dissolved in Phosphate buffer (200mM, p^H 7.4)
- 0.3mM of Nitro-blue tetrazolium(NBT) was dissolved in Sodium carbonate buffer (100mM, p^H 10.35)

3.4 ESTIMATION OF TOTAL PHENOLIC CONTENT

Reagents required

- Distilled water
- Folin's reagent
- Sodium carbonate

3.5 THIN LAYER CHROMATOGRAPHY (TLC) AND PREPARATIVE THIN LAYER CHROMATOGRAPHY (PTLC)

Reagents Required

- Silica gel
- Ethyl acetate
- Formic acid
- Acetic acid
- Acetone
- Distilled water

REFERENCES

- Aarti Narkhede, Pallavi Nirmal, Rashmi Tupe, Omkar Kulkarni, Abhay Harsulkar, Suresh Jagtap '*In vitro* Antioxidant, Antiglycation and a- amylase inhibitory potential of *Eulophia ochreata* L. Journal of Pharmacy Research, Vol.5(5), pp.2532-2537, 2012.
- Ali Ghasemzadeh, Hawa Z. E. Jaafar and Asmah Rahmat 'Effects of solvent type on phenolics and flavonoids content and antioxidant activities in two varieties of young ginger (*Zingiber officinale* Roscoe) extracts Journal of Medicinal Plants Research, Vol. 5(7), pp. 1147-1154, 2011.
- Andreas Schieber, Nicolai Berardini and Reinhold Carle 'Identification of Flavonol and Xanthone Glycosides from Mango (*Mangifera indica* L. Cv. "Tommy Atkins") Peels by High-Performance Liquid Chromatography-Electrospray Ionization Mass Spectrometry, Journal of Agricultural and Food Chemistry, Vol. 51, pp. 5006-5011, 2003.
- Agnieszka Troszynska and Ewa Ciska 'Phenolic Compounds of Seed Coats of White and Coloured Varieties of Pea (*Pisum sativum* L.) and Their Total Antioxidant Activity Czech Journal of Food and Science, Vol. 20, pp. 15–22, 2002.
- Arom Jedsadayanmata '*In Vitro* Antiglycation Activity of Arbutin Naresuan University Journal, Vol.13 (2), pp.35-4, 2005.
- Chan S W, Lee C Y, Yap C F, Wan Aida W M and Ho C W (2009) 'Optimisation of extraction conditions for phenolic compounds from limau purut (*Citrus hystrix*) peels International Food Research Journal, Vol.16, pp. 203-213, 2009.
- Chanda S V and Nagani K V 'Antioxidant Capacity of *Manilkara zapota* L. Leaves Extracts Evaluated by Four *in vitro* Methods Nature Science, Vol.8(10), pp.260-266, 2010.
- Daise Lopes-Lutz, Judith Dettmann, Chamila Nimalaratne and Andreas Schieber 'Characterization and Quantification of Polyphenols in Amazon Grape (*Pourouma cecropiifolia* Martius) Molecules, Vol. *15*, pp. 8543-8552, 2010.
- David Vauzour , Ana Rodriguez-Mateos , Giulia Corona , Maria Jose Oruna-Concha and Jeremy P. E. Spencer 'Polyphenols and Human Health: Prevention of Disease and Mechanisms of Action *Nutrients, Vol. 2*(11), pp.1106-1131, 2010.

- De S, Dey Y.N, Ghosh A.K ‘Phytochemical Investigation and Chromatographic evaluation of the different extracts of tuber of *Amorphaphallus paeoniifolius* (Araceae) □ International Journal of Pharmacuetical and Biomedical research, Vol.1(5), pp.150-157, 2010.
- Deena Ramful, Evelyne Tarnus, Philippe Rondeau, Christine Robert Dasilva, Theeshan Bahorun and Emmanuel Bourdon ‘Citrus Fruit Extracts Reduce Advanced Glycation End Products (AGEs)- and H2O2-Induced Oxidative Stress in Human Adipocytes □ Journal of Agriculture and Food Chemistry, Vol.58, pp.11119–11129, 2010.
- Farooq Anwar, Roman Przybylski ‘Effect of solvents extraction on Total Phenolics and Antioxidant activity of extracts from Flaxseeds (*Linum usitatissimum* L.) □, Acta Scitiarium Polonorum, Technol. Aliment., Vol.11(3), pp.293-301, 2012.
- Gambut, A, Capuano, R, L.Lecce, M.G. Fragasso and Moio, L ‘Extraction of phenolic compounds from 'Aglianico' and 'Uva di Troia' grape skins and seeds in model solutions: Influence of ethanol and maceration time□ Vitis, Vol.48 (4), pp.193–200, 2009.
- Ghulam Abbas, Muhammad Shahzad, Muhammad Jawad Hassan, Rasool Bakhash Tareen and Muhammad Iqbal Choudhary ‘Antiglycation, Antioxidant and Anti Lipid Peroxidation Activities of *Microcephala lamelatta* with Low Cytotoxic Effects *In vitro*□ Middle-East Journal of Scientific Research, Vol.11 (6), pp.814-818, 2012.
- Guo GuangWang, Xiao Hua Lu, Wei Li, Xue Zhao and Cui Zhang ‘Protective Effects of Luteolin onDiabetic Nephropathy in STZ-Induced Diabetic Rats□ Evidence-Based Complementary and Alternative Medicine, Vol.10, pp.1-7, 2011
- Hilary King ‘Global Estimates for Prevalence of Diabetes Mellitus and Impaired Glucose Tolerance in Adults□ Diabetes Care, Vol. 16 (1), 1993.
- Javid Hussain, Nargis Jamila, Syed Abdullah Gilani, Ghulam Abbas and Sagheer Ahmed ‘Platelet aggregation, antiglycation, cytotoxic, phytotoxic and antimicrobial activities of extracts of *Nepeta juncea*□ African Journal of Biotechnology, Vol. 8 (6), pp. 935-940, 2009.
- Javid Hussain, Riaz Ullah, Farman Ullah Khan, Zia Muhammad, Naeem Khan, Najeeb ur Rehman and 2Hidayat Hussain ‘Antiglycation and Antimicrobial Activities of the Crude Extract *of Phlomis bracteosa*□ American-Eurasian J. Agric. & Environ. Sci, Vol.7 (6), pp.634-636, 2010.
- Jerneja Jakopic, Robert Veberic, Franci Stampar ‘Extraction of phenolic compounds from green walnut fruits in different solvents□, Acta agriculturae Slovenica, Vol. 1, pp. 93, 2009.
- Jitender Kumar ‘Evaluation of Flavonoid Content□ International Research Journal, Vol.2 (6), pp. 27-31, 2009.
- Kumar, A, Illavarasan, R, Jayachandran, T, Deecaraman, M, Aravindan, P, Padmanabhan, N, Krishnan, M.R.V ‘Anti-Diabetic activity of *Syzygium cumini* and its isolated compounds against Streptozotocin-induced diabetic rats □ Journal of Medicinal Plant Research, Vol.2(9), pp.246-249,2008.
- Lolita Tomsone, Zanda Kruma, Ruta Galoburda ‘Comparison of Different Solvents and Extraction Methods for Isolation of Phenolic Compounds from Horseradish Roots (*Armoracia rusticana*) □ World Academy of Science, Engineering and Technology, Vol. 64, pp. 903-908, 2012.
- Long-Ze Lin, James M. Harnly ‘Identification of the phenolic components of chrysanthemum flower (Chrysanthemum morifolium Ramat)□ Food Chemistry, Vol. 120, pp.319–326, 2010.
- Louis-Philippe Beaulieu1, Cory S. Harris1, Ammar Saleem, Alain Cuerrier, Pierre S. Haddad, Louis C. Martineau, Steffany A.L. Bennett, John T. Arnason ‘Inhibitory Effect of the Cree Traditional Medicine Wiishichimanaanh (*Vaccinium vitis-idaea*) on Advanced Glycation Endproduct Formation: Identifi cation of Active Principles□ Physiotherapy Research, Vol.24, pp.741–747, 2010.
- Mahesh Chand Meena and Vidhya Patni ‘Isolation and Identification of Flavonoid "Quercetin" from Citrullus colocynthis (Linn.) Schrad□ Asian J. of Exp. Sci., Vol 22 (1), pp.137-142, 2008.
- Mala Majumdar and Prachi S. Parihar ‘Antibacterial, antioxidant and antiglycation potential of *Costus pictus* from Southern region, India□ Asian Journal of Plant Science and Research, Vol.2 (2), pp.95-101, 2012.
- Malgorzata Materska , ‘Quercetin and its derivatives: Chemical structure and bioactivity-a review, Polish of Journal of Food and Nutrition sciences, Vol. 58, pp. 407-413, 2008.
- Mary C. Wells-Knecht, Timothy J. Lyons, David R. McCance, Suzanne R. Thorpe and John W. Baynes ‘Age-dependent Increase in Ortho-Tyrosine and Methionine Sulfoxide in Human Skin Collagen Is Not Accelerated in Diabetes-Evidence against a Generalized Increase in Oxidative Stress in Diabetes□ The American Society for Clinical Investigation, Vol.100(4), pp. 839–846, 1997.
- McIntosh T S, Davis H M, and Matthews D E ‘A Liquid Chromatography±Mass Spectrometry Method to Measure Stable Isotopic Tracer Enrichments of Glycerol and Glucose in Human Serum□ Analytical Biochemistry, Vol.300, pp.163–169, 2012.
- Milan Stefek ‘Natural flavonoids as potential multifunctional agents in prevention of diabetic cataract□ Interdisciplinary Toxicology, Vol.4(2), pp.69–77, 2011.
- Mitchel Linscheid and David G Westmoreland ‘Application of Liquid Chromatography-Mass Spectrometry□ Pure & Applied Chemistry, Vol. 66(9), pp. 1913-1930, 1994.
- Mohammad A, Bhawani S A and Sharma S ‘Analysis of Herbal Products by Thin-layer Chromatography: A Review□ International Journal of Pharma and Biosciences, Vol. 1(2), pp.1-50, 2010.
- Mohammad Ali Esmaeili, Mohammad Reza Kanani, Ali Sonboli ‘*Salvia reuterana* Extract Prevents Formation of Advanced Glycation End Products: An *In Vitro* Study□ Iranian Journal of Pharmaceutical Sciences, Vol. 6(1), pp.33-50, 2010.
- Nasapon Povichit, Ampai Phrutivorapongkul, Maitree Suttajit and Pimporn Leelapornpisid ‘Antiglycation and antioxidant activities of oxyresveratrol extracted from the heartwood of *Artocarpus lakoocha Roxb.* □ Maejo International Journal of Science and Technology, Vol. 4(03), pp.454-461, 2010.
- Nawal Al-Musayeib, Shagufta Perveen, Itrat Fatima, Muhammad Nasir and Ajaz Hussain ‘Antioxidant, Anti-Glycation and Anti-Inflammatory Activities of Phenolic Constituents from *Cordia sinensis*□ Molecules, Vol.*16*, pp.10214-10226, 2011.
- Nicolas Rispail, Phillip Morris, Judith Webb, K ‘Phenolic compounds: Extraction and analysis□ *Lotus japonicus* Handbook, pp.349-355, 2005.
- Patel, DK, Kumar, R, Laloo, D, Hemalatha, S ‘Natural medicines from plant source used for therapy of diabetes mellitus: An overview of its pharmacological aspects□ Asian Pacific Journal of Tropical Disease, pp.239-250, 2012.
- Peer Basha D, Katikala Prasanth Kumar, Bulusu Bhanu Teja, Mannam Subbarao ‘Antidiabetic activity on extracts of *Mangifera indica* in Alloxan monohydrate induced diabetic rats □ Drug Invention Today, Vol.3(7), pp.165-168, 2011.
- Prashant Tiwari, Bimlesh Kumar, Mandeep Kaur, Gurpreet Kaur, Harleen Kaur ‘Phytochemical screening and extraction:a review□ Internationale Phermacuetica Sciencia, Vol.1 (1),pp. 98-106, 2011.
- Ramesh Petchi R, Parasuraman S, Vijaya C, Girish Darwhekar and Devika GS ‘Anti-diabetic effect of Kernel seeds extract of *Mangifera indica* (Anarcadiaceae) □ International journal of Pharma and Bioscience, Vol.2(1), pp.385-393, 2011.
- Robert L Taylor and Ravinder J Singh ‘Validation of Liquid Chromatography–Tandem Mass Spectrometry Method for Analysis of Urinary Conjugated Metanephrine and Normetanephrine for Screening of Pheochromocytoma□ Clinical Chemistry vol. 48(3), pp. 533-539, 2002.
- Rosa Martha Perez Gutierrez, Luis B Flores Cotera and Adriana Maria Neira Gonzalez ‘Evaluation of the Antioxidant and Anti-glication effects of the Hexane Extract from *Piper auritum* Leaves *in Vitro* and Beneficial Activity on Oxidative Stress and Advanced Glycation End-Product-Mediated Renal Injury in Streptozotocin-Treated Diabetic Rats□ Molecules, Vol.17, pp.11897-11919, 2012.
- Russel V Lenth ‘Responce-Surface Methodology in R, using RSM□ Journal of Statistical Software, Vol.32 (7), pp.1-17, 2009.
- Samina Kousar, Munir Ahmad Sheikh, Muhammad Asghar ‘Antiglycation activity of Thiamin-HCl and Benfoitiamine in diabetic condition□ Journal of Pakistan Medical Association, Vol. 62(10), pp. 1033-1038, 2012.
- Seyed Abdul Majid Ayatollahi, Farzad Kobarfard, Jinous Asgarpanah and Muhammad Iqbal Choudhary ‘Antiglycation Activity of *Otostegia persica* (Burm.)Boiss□ African Journal of Biotechnology, Vol. 9(24), pp. 3645-3648, 2010.
- Sheng-Chuan Hsi, Yuan-Ping Kao, Pao-Yuan Wang, Hong-Ming Chao, Chung-Hsiung Huang, Hang-Seng Liu, Li-Jane Shih, Johannes Scheng-Ming Tschen, Ching-Ling Lin and Yung-Hsi Kao ‘Grape Seed Procyanidins Improve Diabetic Symptoms in Mice with Streptozotocin-Induced Diabetes □ The Open Physiology Journal, Vol.2, pp. 6-13, 2009.
- Sourav Mukherjee, Devyani Phatak, Juhi Parikh, Suresh Jagtap, Shamim Shaikh, Rashmi Tupe ‘Antiglycation and antioxidant activity of a rare medicinal orchid *Dendrobium aqueum Lindl.*□ Medicinal Chemistry & Drug Discovery, Vol.2 (2), pp.46-54, 2012.

- Sumaira Naeem, Muhammad Ali and Asif Mahmood 'Optimization of extraction conditions for the extraction of phenolic compounds from *Moringa oleifera* leaves Pakistan Journal of Pharmacuetical Science, Vol.25(3), pp.535-541, 2012.
- Sunitha Singh 'The genetics of Type 2 diabetes mellitus:a review Journal of Scientific Research, Vol.55, pp.35-48, 2011.
- Wannaporn Suwannaphet, Aramsri Meeprom, Sirintorn Yibchok-Anun, Sirichai Adisakwattana 'Preventive effect of grape seed extract against high-fructose diet-induced insulin resistance and oxidative stress in rats Food and Chemical Toxicology, Vol.48, pp.1853–1857, 2010.
- Xiuzhen Han, Tao Shen and Hongxiang Lou 'Dietary Polyphenols and Their Biological Significance International Journal of Molecular Sciences, Vol.8, pp.950-988, 2007.
- Yoshikazu Yonei, Ryo Miyazaki, Yoko Takahashi, Hozumi Takahashi, Keitaro Nomoto Masayuki Yagi, Hiroshige Kawai, Motoki Kubo, Nobuyasu Matsuura 'Anti-Glycation Effect of Mixed Herbal Extract in Individuals with Pre-Diabetes Mellitus:A Double-Blind, Placebo-Controlled, Parallel Group Study Japanese Society of Anti-Aging Medicine, Vol. 7 (5), pp.26-35, 2010.
- Youssef El Hajj, Nicolas Louka1, Catherine Nguyen, Richard G. Maroun 'Low Cost Process for Phenolic Compounds Extraction from Cabernet Sauvignon Grapes (*Vitis vinifera* L. cv. Cabernet Sauvignon). Optimization by Response Surface Methodology Food and Nutrition Sciences, Vol. 3, pp.89-103, 2012.
- Yun Jeong Hong, Tomas-Barberan F A, Adel A Kader and Alyson E Mitchell ' The Flavonoid Glycosides and Procyanidin Composition of Deglet Noor Dates (Phoenix dactylifera) Journal of Agricultural and Food Chemistry, Vol. 54, pp. 2405-2411, 2006.
- Yun-Fang Chen, Hsiao-Yuh Roan, Chong-Kuei Lii, Yuan-Ching Huang, and Tsu-Shing Wang 'Relationship between antioxidant and antiglycation ability of saponins, polyphenols, and polysaccharides in Chinese herbal medicines used to treat diabetes, Journal of Medicinal Plants Research, Vol. 5(11), pp. 2322-2331, 2011.
- www.patient.co.uk/doctor/diabetes-mellitus
- https://apps.who.int/inf-fs/en/fact138.html
- www.diabeteslibrary.org/View.aspx?url=Diabetes_Mellitus_Definition
- http://diabetes.webmd.com/types-of-diabetes-mellitus
- www.medbio.info_horn_PDF files_insulin's mechanism of action.pdf
- macro.lsu.edu/how to/solvents/Polarity index.htm
- www.dionex.com 'Extraction of Phenolic Acids from Plant Tissue Using Accelerated Solvent Extraction (ASE) Application note: 357

List of publications

Presented a paper in "International conference on Bioengineering, a special focus on bioproducts in disease management" on the topic '*In-vitro* antiglycation activity of crude extracts from some selected Indian fruit seeds ', Rajalakshmi college of Engineering, Chennai on January 02/01/2013 to 04/01/2012.

SHAKTHI DEVE.A

M.Tech BIOTECHNOLOGY

Education

2011 – 13	**Kumaraguru College of Technology, Coimbatore.** Autonomous, Anna University, Coimbatore M.Tech Biotechnology. - 8.41*
2007 – 11	**Periyar Maniammai College of Technology for Women, Thanjavur** Anna University, Trichirapalli B.Tech Biotechnology, - 73.92%
2005 – 07	**Cluny Matriculation Higher Secondary School, Salem** Matriculation - 76.75%
2005	**Cluny Matriculation Higher Secondary School, Salem** Matriculation - 83.36%

Skills

Areas of Interest	Stem cells, Cancer Biology, Immunology, Animal Biotechnology
Programming	Familiar with C programming
Computer Knowledge	Fluent with computers having working knowledge of Computer Operating System, Windows 7, Microsoft office (Word, excel, presentation)
Certifications	Diploma in Food and Nutrition, Indira Gandhi Open National University for Distant Education, New Delhi, India
Technical skills	PCR, Chromatography, Electrophoresis, Analytical Techniques

In Plant Training

In plant training in Microcore Research Laboratories, India Pvt Ltd in Research and development Department during 21 Nov,2009 to 30 Nov,2009

Projects

July,2012 **Ongoing project :** Identification and isolation of polyphenols as antiglycative agents from some selected Indian fruit seeds
Guided by: Dr.T.Sathishkumar, Assistant Professor (SRG), Kumaraguru College of Technology, Coimbatore.

April,2011 **Final Year Project:** Comparison of isolation and characterization of Lineage positive and lineage negative population from Bone marrow using Fluorescence Activated Cell Sorter (FACS) and Magnetic Activated Cell Sorter (Macs), with Stem cell Culture Department, Rigid Healthcare Pvt Ltd, Chennai, India.
Guided by: Dr.A.R.Elizabeth, Professor, Periyar Maniammai University, Tanjore.

Papers and Workshops

Jan 2013 Organized GENESPIRE '13-National level Students' Technical Symposium, Department of Biotechnology, Kumaraguru College of Technology, Coimbatore.

Jan 2013 Presented a paper in "**International conference on Bioengineering, a special focus on bioproducts in disease management**" on the topic '***In-vitro* antiglycation activity of crude extracts from some selected Indian fruit seeds ',** Rajalakshmi college of Engineering, Chennai.

Jan 2012 Organized GENESPIRE '12-National level Students' Technical Symposium, Department of Biotechnology, Kumaraguru College of Technology, Coimbatore.

Dec 2011 Participated in Seminar on "Postharvest Technology and Cool Chain Management", Vanavarayar Institute of Agriculture, Pollachi and Writtle College, UK.

Aug 2009 Presented a **research brief** on various **Food processing techniques** in the International conference on Food & technology and workshop on HACCP by Indian Institute of crop processing technology

Aug 2009 Participated in Research Awareness Seminar by Associate dean of research Dr. Namas Chandra(College of engineering, University of Nebraska, Lincoln, Nebraska, USA)

Mar 2009 Participated in Workshop on Strategic Planning by Dr.Chris Kaufman (President, Agovia Consulting Inc., Seattle, USA)

July 2008 Presented a poster on(Sunscreens and Cosmetics Containing Manganese Doped Titanium oxide Nano Particles) in the GENOMERA'08 – National seminar on NanoBiotechnology by Department of Science & Technology, India

Personal Information

Date of Birth : 2nd Aug,1989
Father's Name : Mr. A.Asaithambi
Mother's Name : Mrs. K.Padmavathi
Address : 83,Mariamman Kovil Street,
Peramanoor,
Salem-636007
Mobile No. : 9597250881
e – mail ID : pksshakthi@gmail.com

Languages Known : Tamil, English and Hindi
Passport : G0839140 valid up to 2016

Declaration: The information furnished above is correct to the best of my knowledge.

Date: 02.05.2013 (Shakthi Deve.A)

Positions of Responsibility

- Aug 2012 Member of Association of Biotechnology, Kumaraguru College Of Technology, Coimbatore
- Aug 2010 **Organizer** of GENOMERA'10-National seminar on **Plant and Human Health** by AYUSH, Ministry of health and Family welfare, New Delhi, India
- Aug 2009 Played a lead role in a team of ten, in **Organizing**, GENOMERA'09 – National seminar on **Herbal Plant Biotechnology** by AYUSH, Ministry of health and Family welfare, New Delhi, India
- Member of Basket Ball Club during under graduation.
- Member of National Service Scheme.

Achievements and Awards

- **Outstanding** performance in exams conducted by Hindi Prachar Sabha.
- School level Participation in District Level **Basket Ball** Tournament
- Awarded prize for Poster Presentation on various **Food processing techniques** in the International conference on Food & technology and workshop on HACCP by Indian Institute of crop processing technology

Interests

- Reading books
- Cooking