ANTIDIABETIC SCREENING AND PHYTOCHEMICAL INVESTIGATION OF SELECTED MEDICINAL PLANTS

THESIS SUBMITTED TO
THE TAMILNADU DR. M. G. R. MEDICAL UNIVERSITY, CHENNAI,
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OF
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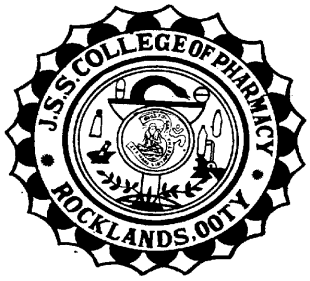
IN
PHARMACEUTICAL SCIENCES
BY

LAKSHMINARASIMHAIAH

UNDER THE GUIDANCE OF

DR. M. J. N. CHANDRASEKAR



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CERTIFICATE

This is to certify that the thesis entitled **"Antidiabetic screening and phytochemical investigation of selected medicinal plants"** submitted by **Mr. Lakshminarasimhaiah**, to The Tamilnadu DR. M. G. R. Medical University, Chennai, for the award of the degree of Doctor of Philosophy in Pharmaceutical Sciences, is a record of the independent research work carried out by him at J. S. S. College of Pharmacy, Ootacamund, under my supervision, during 2007-2012. I also certify that this thesis or any part thereof has not formed the basis for the award of any other research degree, of this or any other University, previously.

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CERTIFICATE

This is to certify that the thesis entitled **"Antidiabetic screening and phytochemical investigation of selected medicinal plants"** submitted by **Mr. Lakshminarasimhaiah,** to The Tamilnadu Dr. M. G. R. Medical, University, Chennai, for the award of the Degree of Doctor of Philosophy in Pharmaceutical Sciences, is based on the research work carried out by him under the supervision of **Dr. M. J. N. Chandrasekar,** Professor, J. S. S. College of Pharmacy, Ootacamund. The thesis or any part thereof has not formed the basis for the award of any other research degree, of this or any other University, previously.

Principal

DECLARATION

I hereby declare that the thesis entitled **"Antidiabetic screening and phytochemical investigation of selected medicinal plants"** submitted by me to The Tamilnadu Dr. M. G. R. Medical University, Chennai, for the award of the Degree of Doctor of Philosophy in Pharmaceutical Sciences, is the result of my original and independent research work carried out at Department of Pharmaceutical Chemistry, J. S. S. College of Pharmacy, Ootacamund, under the supervision of **Dr. M. J. N. Chandrasekar**, Professor, J. S. S. College of Pharmacy, Ootacamund. The thesis or any part thereof has not formed the basis for the award of any degree, diploma, associateship, fellowship or any other similar title, of this or any other University, previously.

Date: 10.04.2012 **Lakshminarasimhaiah**

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Lakshminarasimhaiah

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1.INTRODUCTION

1.1 Drug Discovery

Drug discovery is the identification of novel active chemical compounds. The drug discovery is made through the observations of biological effects of new or existing natural products from micro organisms, plants etc. The drug discovery is also bound to therapeutic targets such as enzymes, receptors etc. Pharmacophore approaches have become one of the major tools in drug discovery. The ligand based and structure based methods have been developed for improved pharmacophore modeling [1, 2]. The drug target is the naturally existing cellular or molecular structure involved in the pathology of interest that the drug in development is meant to act on. The drug target may be a established target or new target. The process of finding a new drug against a chosen target for a particular disease usually involves high-throughput screening. Two major approaches exist for the finding of new bioactive chemical compound from natural sources. Screening the chemical compounds for biological activity and structure elucidation of chemical compounds by NMR, Mass spectroscopy [3].

In the post genomic era, pharmaceutical researchers are evaluating vast numbers of protein sequences to formulate novel strategies for identifying valid targets and discovering leads against them. Modern drug discovery often involves screening small molecules for their ability to bind to a preselected protein target. Drug discovery can also involve screening small molecules for their ability to modulate biological pathways in cells or organisms, without regard to any particular protein target. Thus the establishment of various techniques of genomic sciences such as rapid DNA sequencing, together with combinatorial chemistry, cell based assays and automated high throughput screening (HTS) has led to a new concept of drug discovery. In this concept, interaction between biologists and chemists, as well as scientific reasoning has been replaced by a very high number of samples processed. With rapid industrialization, an HTS system has been developed to screen hundreds of thousands of chemical compounds in a short amount of time. HTS was created in the early 1990 for rapid screening of large number of extracts or compounds [4, 5]. This requires the identification of disease specific targets by basic research or by genomic approach, which is

used to develop a bioassay used in the HTS system. About 50 million screening tests have been conducted so far using different molecules, different concentrations and different bioassays. These technologies generated vast amounts of information on natural products obtained from plants and microorganisms.

Plant cells produce two types of metabolites. Primary metabolites are involved directly in growth and metabolism, viz. carbohydrates, lipids and proteins. Primary metabolites are produced as a result of photosynthesis and are additionally involved in cell component synthesis. Most natural products are compounds derived from primary metabolites such as amino acids, carbohydrates and fatty acids and are generally categorized as secondary metabolites. Secondary metabolites are considered products of primary metabolism and are generally not involved in metabolic activity, viz. alkaloids, phenolics, essential oils, terpenes, sterols, flavonoids, lignins, tannins, glycosides, etc. These secondary metabolites are the major source of pharmaceuticals, food additives, fragrances and pesticides [6].

Primary metabolites obtained from higher plants for commercial use are high volume, low value bulk chemicals. They are primarily used as industrial raw materials, foods or food additives such as vegetable oils, carbohydrates and proteins. Medicinal plants are rich in secondary plant products. These secondary metabolites exert a profound physiological effect on mammalian systems. Thus they are known as the active principles of plants. Besides secondary plant products, several primary metabolites exert strong physiological effects. Primary metabolites exert a strong physiological effect include certain antibiotics, vaccines and several polysaccharides acting as hormones [7, 8, 9]. Secondary metabolites of plants are given below.

According to Pelletier, an alkaloid is a cyclic organic compound containing nitrogen in a negative oxidation state which is limited distribution among living organisms. Sometimes it is not possible to draw a clear line between true alkaloids and certain plant bases. Simple bases such as methylamine, trimethylamine and other straight chain alkylamines are not considered alkaloids. Other compounds such as betaines, choline and muscarine are also excluded from alkaloids by some experts. Some authorities even exclude the

phenylalkylamines, such as β-phenylethylamine, dopamine, ephedrine, mescaline and tryptamine [10, 11, 12]. Widely distributed vitamin B_1 is not categorized as an alkaloid even though it contains a nitrogen in heterocycle and has physiological activity. Similarly purine based compounds caffeine, theophylline and theobromine are also excluded from alkaloids as they are not derived from amino acids. A neutral compound such as colchicine from autumn crocus is an alkaloid, in which nitrogen present in amide group. Other examples of neutral compounds such as alkaloids are piperine from black pepper, betaine and trigonelline. The potent physiological activity of many alkaloids has also led to their use as pharmaceuticals, stimulants, narcotics and poisons. Alkaloids currently in clinical use include the analgesics morphine and codeine, the anticancer agent vinblastine, the gout suppressant colchicines, muscle relaxant tubocurarine, antiarrythemic ajmalicine, antibiotic sanguinarine and sedative scopolamine. Piperidine alkaloids such as coniceine, coniine and N-methyl coniine are present in Conium maculatum. The most commonly occurring compound is trigonelline, which is present in Trigonella foenum-graecum. Anticholinergic alkaloids hyoscyamine, atropine and hyoscine are found principally in plants of the family Solanaceae. Nicotine and tropane alkaloids are formed in the roots and transported to the aerial parts of the plant. The tropane alkaloids possess an 8-azabicyclo octane nucleus and are found in the plants of three families, Solanaceae, Erythroxylaceae and Convolvulaceae [13, 14].

Simple phenolic compounds have at least one hydroxyl group attached to an aromatic ring. Most compounds having a C_6C_1 carbon skeleton, usually with a carbonyl group attached to aromatic ring. Simple phenylpropanoids are defined as secondary metabolites derived from phenylalanine, having a C_6C_3 carbon skeleton, and most of them are phenolic acids e.g. cinnamic acid, o-coumaric acid, p-coumaric acid, caffeic acid and ferulic acid [15, 16, 17]. A simple phenylpropanoid can conjugate with an intermediate from the shikimic acid pathway, such as quinic acid to form compounds like chlorogenic acid. Phenolic compounds having a $C_6C_3C_6$ carbon skeleton include flavonoids and isoflavonoids. Resveratrol is an oligomeric polyphenol found as dimer, trimer and tetramer in the families Vitaceae, Dipterocarpaceae, Cyperaceae, Gnetaceae and Leguminosae. Resveratrol is synthesized from phenylalanine, mediated by the enzyme stilbenes synthase, while chalcone synthase converts phenylalanine

into flavonoids. Resveratrol is implicated in the prevention of cancer and cardiovascular diseases in vasoprotection and neuroprotection. The phenolic group includes metabolites derived from the condensation of acetate units, those produced by the modification of aromatic amino acids, flavonoids, isoflavonoids and tannins. The phenolics derived from aromatic amino acids and their precursors are just some of the very wide range of compounds derived from shikimic acid. A phenyl group having three carbon side chains is known as a phenylpropanoid, such as hydroxycoumarins, phenylpropenes and lignans. The phenylpropenes are important components of many essential oils, e.g. eugenol in clove oil and anethole and myristicin in nutmeg.

Flavonoids have two benzene rings attached by a propane unit and are derived from flavones. They are found throughout the plant kingdom, whereas isoflavonoids are more restricted in distribution, and are present in the family Fabaceae, in which they are widely distributed and function as antimicrobial, anti-insect compounds, as an inducer of nodulation genes of symbiotic Rhizobium bacteria or as allelopathic agents. Flavonoids are brightly coloured compounds generally present in plants as their glycosides. Different classes within this group differ by additional oxygen containing heterocyclic rings and hydroxyl groups and include the chalcones, flavones, flavonols, anthocyanins and isoflavones [18]. Anthocyanins impart red and blue pigment to flowers and fruits and can make up as much as 30% of the dry weight of some flowers. Flavanones, flavonols and anthocyanins normally exist as their glycosides. The isoflavonoids are rearranged flavonoids, in which this rearrangement is brought about by a cytochrome P-450 dependent enzyme which transforms the flavanones, liquiritigenin or naringenin into isoflavones daidzein or genistein, respectively. Isoflavones exhibit estrogenic, antiangeogenic, antioxidant and anticancer properties.

Terpenes are unique group of hydrocarbon based natural products that possess a structure that are derived from isoprenes, giving rise to structures that may be divided into isopentane units [19]. Compounds having 3-isoprene units are called sesquiterpenes, exist in aliphatic, bicyclic and tricyclic frameworks. A member of this series, farnesol is a key intermediate in terpenoid biosynthesis. Arteether is a sesquiterpene lactone isolated from *Artemisia annua* and currently used as an antimalarial drug. The diterpenes are not considered essential oils

and constitute a component of plant resins because of their higher boiling point. These are composed of four isoprene units. Gibberellic acid and taxol are diterpenes. Triterpenes are composed of six isoprene units and are biosynthetically derived from squalene. These are high melting point, colourless solids and constitute a component of resins, cork and cutin. Triterpenoids produce several pharmacologically active groups such as steroids, saponins and cardiac glycosides. Azadirachtin is obtained from seeds of *Azadirachta indica*. Other triterpenoids include the limonins and the cucurbitacins, which are potent insect steroid hormone antagonists. Steroids are modified triterpenes and have profound importance as hormones, coenzymes and provitamins in animals. Many progesterones are derived semisynthetically from diosgenin. Saponins are C_{27} steroids widely distributed in monocot families like Liliaceae, Amaryllidaceae and Dioscoreaceae, and in dicot families, e.g. Scrophulariaceae and Solanaceae. Saponins are composed of two parts: the glycon and aglycon. Commercially important preparations based on saponins include sarsaparilla root, licorice, ivy leaves, primula root and ginseng.

Natural products including plants, animals and minerals have been the basis of treatment of human diseases. History of medicines dates back practically to the existence of human civilization [20]. The history of medicines includes many ludicrous therapies. The future of natural product drug discovery will be more holistic, personalized and involve wise use of ancient and modern therapeutic skills in a complimentary manner so that maximum benefits can be accrued to the patients and the community. Herbal drug development includes various steps, starting from raw materials data, correct identification, pharmacognostic and chemical quality standardization, safety and preclinical pharmacology, clinical pharmacology and controlled clinical trials. Herbal medicines were developed at times of limited access to technologically variable norms of standardization. Advanced synthetic organic chemistry helps to the identification of many chemical molecules, it leads to the development of novel compounds.

Natural products produced by plants, fungi, bacteria, protozoans, insects and animals have been isolated as biologically active pharmacophores. Natural products are likely to continue to be sources of new commercially viable drug leads. The chemical novelty associated with

natural products is higher than that of any other source. Natural products are traditional, empirical and molecular [21]. The traditional approach makes the use of material that has been found by trial and error over many years in different cultures and systems of medicines. Examples include drugs such as morphine, quinine and ephedrine. The empirical approach builds on an understanding of a relevant physiological process and develop therapeutic agent from a naturally occurring lead molecule. Examples include tubocurarine and other muscle relaxants, propranolol and other β-adrenergic antagonists, cimetidine and H_2 receptor antagonist. The molecular approach is based on the availability or understanding of a molecular target for the medicinal agent. The development of molecular biological techniques and advances in genomics, the majority of drug discovery is based on the molecular approach [22].

Plant products are rich source of lead molecules in drug discovery. According to the collected statistics, drug developed between1981-2002 showed that natural product or natural product derived drugs comprised 28% of all new chemical entities launched in the market [23]. Plant products are important source of new drugs and are also good lead compounds suitable for further modification during drug development. Natural products and related drugs are used to treat 87% of all categorized diseases [24]. The search for novel drug suggests the utilization of plants as potential source and to increase the isolation of novel compounds from plant source. The secondary metabolites from natural products are showing more drug likeness and biologically friendliness than total synthetic molecules.

Over 120 pharmaceutical products in use today are obtained from the plants. A large number of therapeutic activities are mediated by these drugs, and a host of drugs in use are still obtained from plants in which they are synthesized. Examples include, cardiotonic glycosides (Digitalis glycosides), anticholinergics (Tropane alkaloids), analgesics and antitussives (Opium alkaloids), antihypertensives (reserpine), cholinergics (physostigmine, pilocarpine), antimalarials (cinchona alkaloids), antigout (colchicines), anesthetic (cocaine), skeletal muscle relaxant (tubocurarine) and anticancer agents (paclitaxel, vincrystine, teniposide and analogues of camptothecin).

Analysis of the number and sources of anticancer and anti-infective agents, reviewed mainly in Annual Reports of Medicinal Chemistry from 1984 to 1995, indicates that over 60% of the approved drugs and pre-NDA candidates (for the period 1989-1995), excluding biologics, developed in these disease areas are of natural origin. According to Newmann et al., 2003, during the period 1981-2002 a vast majority of New Chemical Entities is from natural products source. Thus natural products have been playing an invaluable role in the drug discovery process, particularly in the areas of diabetes, cancer and infectious diseases.

Plants have thus been a prime source of highly effective conventional drugs for the treatment of diabetes. While the actual compounds isolated from plants frequently may not serve as drugs, they provide leads for the development of potential novel agents. As new technologies are developed, some of the agents which failed earlier in clinical studies are now stimulating renewed interest. The appreciation of the significance of natural products as sources for structurally novel and mechanistically unique drugs and the presence of an enormous biodiversity of India, prompted the writer's interest in evaluating the traditional medicinal plants for their antioxidant and antidiabetic properties.

The chemical, pharmacological and clinical studies of the traditional medicines, which were derived from plants are the most early medicines such as aspirin, digitoxin, morphine, quinine and pilocarpine. High-throughput screening and mechanism based screening has become mainstay in drug discovery. The mechanism based screening methods included clavulanic acid, mevastatin and amoxicillin [25]. Natural products are source of new drugs for many diseases and natural product derived drugs are well represented in the top 35 worldwide selling ethical drug sales of 2000, 2001 and 2002. The percentage of natural product derived drugs was 40% in 2000 and remained approximately constant at 24% in 2001 and 26% in 2002. Natural products have historically provided many novel drug leads.

The natural product is extracted from the source, concentrated, fractionated and purified, yielding essentially a single biologically active compound. Determination of the molecular formula is done by high resolution mass spectrometry on microgram quantities of material. Combining the tools of high resolution mass spectrometry with two-dimensional NMR

spectroscopy allows structure determination on milligram amount of compound in hours or days [26].

1.2 Herbal Medicine

Man has been using herbs and plant products for combating diseases since times immemorial. The Indian subcontinent is enriched by a variety of flora both aromatic and medicinal plants. This is due to the wide diversity of climatic conditions of India. Numerous types of herbs have been well recognized and catalogued by botanists from Himalaya to Kanyakumari. This extensive flora has been utilized as a source of many drugs in the Indian traditional system of medicine [27].

The WHO is actively encouraging the developing countries to use herbal medicine which they have been traditionally used for centuries. There are 3000 plants have been identified in the forests of India which can be used as medicine. The active ingredients from these plants are worth Rs 2000 crores in the US market. The science of medicine developed around these plants had curative properties. A continued search for medicinal plants during the last several centuries has given rise to long list of plants which are of use in the treatment of diseases and for promoting health. Drugs used in medicine today are either obtained from nature or are of synthetic origin. Natural drugs are obtained from plants, animals, microbes or minerals. The drugs obtained from plants and animals are called drugs of biological origin and produced in the living cells of plants or animals [28].

There are 6000 plant constituents have been isolated and studied. The plants are inexhaustible source of medicine, remains incompletely explored. This unexplored world provides the most challenging aspects of pharmaceutical and medical science to scientists in search for new and more potent drugs with negligible side effects. During the last few decades, tremendous progress has been made in the study of phytochemicals.

Plants have been one of the important source of medicine since the dawn of human civilization. The Chinese drug Mahung was in use for over 5000 years for the treatment of different types of fever and respiratory disorders. Cinchona was in use in Peru in 1825 for

controlling malaria. The tremendous development in the field of synthetic drugs and antibiotics during 21st century, plants still contribute one of the major sources of drugs in modern and traditional medicine throughout the world. One-third of the world's population treat themselves with traditional medicines. Some of the compounds now commonly used in medicine were isolated from plant sources and used in the 19th century. Examples are morphine, quinine, atropine, papaverine, cocaine, digitoxine and pilocarpine. Examples of some important compounds isolated in 20th century include ergotamine, labeline, digoxine, reserpine, tubocurarine, diosgenin, vincrystine and vinblastine. Plants are the important source of a number of well established and important source of drugs. They are also source of chemical intermediates needed for the production of drugs [29].

Before independence of India, the production of plant based drugs in India was confined mainly to cinchona, opium alkaloids, galanicals and tinctures. In the last three decades, bulk production of plant drugs has become an important aspect of the Indian pharmaceutical industry. Some of the drugs which are manufactured today include morphine, codeine, papaverine, thebaine, emetine, quinine, quinidine, digoxine, caffeine, hyoscine, hyoscyamine, atropine, xanthotoxin, sennosides, colchicines, berberine, vinblastine, vincrystine, ergot alkaloids, papaine, nicotine, strychnine, brucine and pyrethroids.

In India, there are about 20 well recognized manufacturers of herbal drugs, 140 medium or small scale manufacturers and about 1200 licensed small manufacturers on record, in addition to many vaidyas having small manufacturing facilities. The estimated current annual production of herbal drugs is around Rs 100 crores. The demand for herbal remedies is ever-increasing. There are 1650 herbal formulations in the Indian market and 540 major plants involved in their formulations. During the last two decades, over 3000 plants have been screened in India for their biological activities. As a result, a number of new drugs have been introduced in clinical practice and some are in advance stages of clinical development. There are well documented scientific data on a good number of medicinal plants that have been investigated.

Herbal medicines are the use of plants and plant extracts as medicines. In 2001 researchers identified122 compounds used in medicine which were derived from ethnomedical plant sources, 80% of these compounds were used in traditional ethnomedical use. Plants have evolved the ability to synthesize chemical compounds that help them to defend against attack from a wide variety of predators such as insects, fungi and herbivorous mammals. Some of these compounds being toxic to plant predators have beneficial effect when used to treat human diseases. People on all continents have used hundreds to thousands of indigenous plants for treatment of ailments since prehistoric times. Medicinal herbs were found in the personal effects of Otzi the iceman, whose body was frozen in the Otztal Alps for more than 5300 years [30].

In Indian Ayurveda medicine has used many herbs such as turmeric, pepper, garlic in 1900 B.C. Many other herbs and minerals used in ayurveda were described by Charaka and Sushruta. Sushruta described 700 medicinal plants, 64 preparations from mineral sources and 57 preparations based on animal sources. Many of the pharmaceuticals currently available to physicians have a long history of use as herbal remedies including opium, aspirin, digitalis and quinine. The WHO estimates that 80 percent of the world's population presently uses herbal medicine for primary health care. Herbal medicines are available in the market from health food stores without prescriptions and are widely used in India, China, USA and all over the world. Herbal products are classified as dietary supplements and are marketed pursuant to the dietary supplements Health and Education act of 1994. The herbal products are regulated differently in other countries. In United Kingdom any product that is not granted a licence as a medical product by Medicine Control Agency is treated as food and no health claim or medical advice can be given on the label. Labeling of herbal products may not actually reflect the content and adverse events or interactions attributed to specific herb [31].

The commonly used many herbal medicines in their irregular, high doses or with other medications in long term are toxic. The toxic effects of herbal medicines range from allergic reactions to cardiovascular, hepatic, renal, neurological and dermatological toxic effects.

Several herbal products lower the seizure threshold maintained by Phenobarbital. Licorice is used as an anti inflammatory herb and also as remedy for gastric and peptic ulcers.

The importance of plants as a source of useful antihypertensive drugs was supported by the isolation of reserpine from *Rauwolfia serpentine*. Veratrum alkaloids are other useful antihypertensive agents obtained from plant source. *Allium sativum, Zingiber officinale* etc., have been mentioned to be useful in cardiovascular ailments in classical textbooks on ancient medicine. Plant products have contributed several novel compounds possessing promising antitumour activity. For example, podophyllotoxin, alpha and beta pelatin were found to be capable of inflicting considerable damage on experimental tumours. Various herbal medicines having a role in the treatment of diabetes have been described in classical ayurvedic literature. Mention has also been made of different plant extracts used for anti-diabetic activity. Quinquefolans A, B and C isolated from *Panax quiquefolin* had a hypoglycemic effect in normal mice. Quinquefolan A on i.p. administration alone, in alloxan induced hyperglycemic mice produced a hypoglycemic effect [32].

Among the several plants investigated for anti-asthmatic effects, saponins isolated from *Clerodendron serratum*, *Gardenia turgida*, *Albizzia lebbek* and *Solanum xanthocarpum* were found to accord protection to sensitized guinea pigs against histamine as well as antigen micro-aerosols. The protective effect of *C. serratum* saponin was found to be associated with the augmentation of anti-allergic activity in the lung tissues. Saponins from *A. lebbek* have also been demonstrated to modulate immune responses through synthesis of reagenic antibodies. The alcoholic extract of Tylophora asthmatica has been reported to prevent egg albumin-induced anaphylaxis in guinea pigs and horse serum-induced bronchoconstriction in sensitized rat lung. The plant saponins from *C. serratum* and *A. lebbek* as well as alkaloidal fraction of *S. xanthocerpum and T. asthmatica* have been shown to protect sensitized mast cells from degranulation on antigen shock, thus confirming the immunosuppressive and membrane stabilizing effect. *T. asthmatica* as well as saponin of *A. lebbek* have also been found to potentiate bronchodilator beta-adrenergic activity, which is considered to be helpful for relieving bronchospasm in asthmatic patients. The anti-allergic action of *O. sanctum* has been found to be associated with significant production of IgE antibodies [33].

Search for a potent hypolipidaemic agent based on ancient insight following the Ayurvedic system, has been rewarding with the isolation of the oleoresin fraction from *Commiphora mukul* and Guggul having hypolipidaemic activity, comparable to Clofibrate with more favourable HDL-LDL cholesterol ratio. It also decreases platelet adhesiveness and increases fibrinolytic activity necessary for the prevention of myocardial infarction. The hypocholesterolaemic effect of Pterocarpus marsupium associated with hypoglycemic activity, is of clinical significance as hypocholesterolaemia is often associated with diabetes.

Medicinal plants commonly included in Ayuveda for liver ailments have drawn much attention as there is no reliable hepato-protective drug available in modern medicine. The hepato-protective effect of some liver protectives like *Picrorhiza kurrooa, T. cordifolia, Tephrosia purpurea* against carbon tetrachloride and galactosamine-induced hepatic injury have been confirmed experimentally by various workers. In biliary ailments, plants such as *Andrographis paniculata, Lyffa ectinata* and *Ficus hispida* have been found to increase bile flow with reduction in serum bilirubin and SGPT levels. *Phyllanthus niruri* and *Eclipta alba* have been reported to eliminate hepatitis B surface antigen [34].

The specific plants to be used and the methods of application for particular ailments were passed down through oral history. Later on, information regarding medicinal plants was recorded in herbals. Historically herbal drugs were used as tinctures, poultices, powders and teas followed by formulations and lastly as pure compounds. Medicinal plants or their extracts have been used by humans since time immemorial for different ailments and have provided valuable drugs such as analgesics (morphine), antitussive (codeine), antihypertensives (reserpine), cardiotonics (digoxin), antineoplastic (vinblastine and taxol) and antimalarials (quinine and artemisinine). Some of the plants which continue to be used from Mesopotamian civilization to this day are Cedrus spp, *Cupressus sempervirens, Glycirrhiza glabra, Commiphora wightii* and *Papaver somniferum*. About two dozen new drugs derived from natural sources were approved by the FDA and introduced to the market during the period 2000-2005 and include drugs for cancer, neurological, cardiovascular, metabolic and immunological diseases, and genetic disorders. Seven plant derived drugs

currently used clinically for various types of cancers are taxol from Taxus species, vinblastine and vincrystine from *Catharanthus roseus*, topotecan and irinotecan from *Camptotheca accuminata*, and etoposide and teniposide from *Podophyllum peltatum*. The herbal drugs are collected from the wild and few species are cultivated. Overexploitation of plants, particularly when roots, tubers and bark are used for commercial purposes, has endangered the 4000 to 10000 species of medicinal plants. To counter overexploitation of natural resources and the consequent threats to biodiversity, alternative biotechnological methods and sustainable practices have been recommended. The world organizations and governments have established guidelines for the collection and utilization of medicinal plants [35].

1.3 Antidiabetic herbal drugs

Anti-diabetic herbs are useful to reduce high blood glucose levels. These herbs are useful depending on the nature of the diabetes, age, stress of the person and many other factors. Natural products have played a critical role in the identification of numerous anti-diabetic medicines. Plants are major source of anti-diabetic drugs and many of the drugs are derived directly or indirectly from plants. The ethno botanical information reports nearly 800 plants have anti-diabetic activity. The synthetic drugs widely used for hypoglycemic activity came from traditional origin. Thus plants are the pioneer source of anti-diabetic drugs. The advancement in synthetic organic chemistry and combinatorial chemistry strategies has enabled the synthesis of natural product type of compounds. The combination of these approaches are improving the desired biological properties of natural products as well as identification of novel compounds for diabetes.

Many herbal extracts or derivatives have been documented in traditional Chinese medicine as anti-diabetic drugs having clinical effectiveness in treating sugar imbalances in diabetes mellitus [36, 37]. The herbal medicines listed in Table 1 are used for the treatment of diabetes in traditional Chinese medicine. It is estimated that more than 200 species of plants exhibit hypoglycemic properties, including many common plants such as pumpkin, wheat,

celery, wax guard, lotus root and bitter melon. The hundreds of herbs and formulas reported to have been used in traditional Chinese medicine for treatment of diabetes mellitus. Many Chinese herbs contain polysaccharide lower the blood glucose [38].

The ethnobotanical information reports about 800 plants possess antidiabetic potential. Several such herbs have shown antidiabetic activity when assessed using presently available experimental techniques [39]. Among these are alkaloids, glycosides, polysaccharides, peptidoglycans, hypoglycans, guanidine, steroids, carbohydrates, glycopeptides, terpenoids, amino acids and inorganic ions. Some plants with antidiabetic potential are listed in Table 1

Table 1: Traditional medicine of Chinese and Indian antidiabetic herbs

Sl. No	Family	Botanical name
1.	Amaranthaceae	*Achyranthes bidentata*
2.	Scrophulariaceae	*Alisma orientale*
3.	Asparagaceae	*Anemarrhena asphodeloides*
4.	Asparagaceae	*Asparagus cochinchinensis*
5.	Leguminoceae	*Astragaus membranaceus*
6.	Asteraceae	*Atractylodes macrocephala*
7.	Apiaceae	*Bupleurum chinense*
8.	Lauraceae	*Cinnamomum cassia*
9.	Cornaceae	*Cornus officinalis*
10.	Cucurbitaceae	*Cucurbita moschata*
11.	Dioscoreaceae	*Dioscorea opposite*
12.	Rosaceae	*Eriobotrya japonica*
13.	Caprifoliaceae	*Lonicera japonica*
14.	Polygonaceae	*Polygonum multiflorum*
15.	Polyporaceae	*Poria cocos*
16.	Fabaceae	*Pueraria lobata*
17.	Scrophulariaceae	*Rehmannia glutinosa*
18.	Asteraceae	*Artemisia pallens*
19.	Malvaceae	*Bombax ceiba*
20.	Brassicaceae	*Brassica juncea*
21.	Fabaceae	*Caesalpinia bonducella*
22.	Myrtaceae	*Eucalyptus globules*
23.	Myrtaceae	*Eugenia uniflora*

24.	Asclepiadaceae	*Gymnema sylvestre*
25.	Anacardiaceae	*Mangifera indica*
26.	Melastomataceae	*Memecylon umbellatum*
27.	Fabaceae	*Mucuna pruriens*

The indigenous diet may not be useful in lowering the blood glucose to the same extent as insulin and other hypoglycemic agent. But it has some other influences, which may be useful for the management of the disease and its complications. The juices of bitter gourd, decoction of chirata, neem leaves, betel leaves, fenugreek seeds and sada bahar flowers achieve 10-20% lowering of blood glucose. It is useful as supplement to other therapies. Vegetables have antidiabetic potency. Vegetables such as cabbage, capsicum, green leafy vegetables, beans and tubers have shown the hypoglycemic effect in both experimental animals and humans.

1.4 Free radicals

A free radical is an atom or a molecule that contains one or more unpaired electrons [40]. Unpaired electrons alter the chemical reactivity of an atom or molecule; usually make it more reactive than the corresponding non-radical. The actual chemical reactivity of radicals, however, varies enormously. The hydrogen radical, which contain one proton and one electron, is the simplest free radical.

Free radicals in the body are generated by multiple mechanisms and are often initiated by removal of an H atom from other molecules. Living organisms are exposed to electromagnetic radiation from the environment, both natural and from man made sources. Low wavelength electromagnetic radiation (i.e. gamma rays) can split water in the body to generate hydroxyl radicals(OH). Hydroxyl radical has a very short *in vivo* half-life, reacting at its site of formation, usually leaving behind a legacy of free radical chain reactions [41].

The body, through metabolic process, makes an oxygen radical called superoxide (O_2), where the unpaired electron is located on oxygen. Superoxide is made by adding one electron to the oxygen molecule, and is generally poorly reactive [42]. Many molecules in the body react directly with oxygen to make superoxide, including the catecholamines, tetrahydrofolate and some constituents of mitochondrial and other electron transport chains.

Even when this mode of superoxide generation is not available, activated phagocytes generate large amounts of superoxide as part of the mechanism by which foreign organisms are killed. During chronic inflammation, this normal protective mechanism may become damaging.

Another physiological free radical is nitric oxide (NO), which is made by vascular endothelium as a relaxing factor [43]. Nitric oxide has many useful physiological functions, but excess nitric oxide can be toxic. Neither superoxide nor nitric oxide is highly reactive chemically, but under certain circumstances they can generate more reactive toxic products.

When oxygen is reduced in the electron transport chain, oxygen derived free radical intermediates are formed. The O_2 and H_2O_2 intermediates can escape from the system, and in the presence of transition metal ions form the more reactive hydroxyl radicals. While O_2 are toxic to cells, the high reactivity of OH and O_2 renders these activated forms most cytotoxic due to deleterious peroxidation reactions with lipids, proteins and DNA. Lipid peroxidation is an example of this oxidative damage [44]. Free radicals may attack polyunsaturated fatty acids within membranes, forming peroxyl radicals. These newly formed free radicals can then attack adjacent fatty acids within membranes causing a chain reaction of lipid peroxidation. The lipid hydroperoxide end products are also harmful, and may be responsible for some of the overall effect, which can lead to tissue and organ damage.

Antioxidants may be defined as radical scavengers which protect the human body against free radicals that may cause pathological conditions such as ischemia, anaemia, asthma, arthritis, inflammation, neurodegeneration, parkinson's disease, mongolism, ageing and dementias. Flavonoids and flavones are widely distributed secondary metabolites with antioxidant and antiradical properties [45]. Reactive oxygen species (ROS) including superoxide radicals, hydroxyl radicals, singlet oxygen and hydrogen peroxide are often generated as by products of biological reaction or from exogenous factors. In *vivo*, some of these ROS play an important role in cell metabolism including energy production, phagocytosis and intercellular signaling. These ROS produced by sunlight, ultraviolet light, ionizing radiation, chemical reactions and metabolic process have a wide variety of

pathological effects such as DNA damage, carcinogenesis and various degenerative disorders such as cardiovascular diseases, ageing and neurodegenerative diseases [46]. A potent broad spectrum scavenger of these species may serve as a possible preventive intervention for free radical mediated cellular damage and diseases. Recent studies have shown that a number of plant products including polyphenols, terpenes and various plant extracts exerted an antioxidant action. Several medicinal plants have been extensively used in the Indian traditional system of medicine for treatment of number of diseases. Some of these plants have shown potent antioxidant activity.

Oxidative stress is exerted by all peroxides, which can damage cells and tissues, or directly through their more reactive breakdown products such as malonaldehyde and hydroxynonenals [47]. Moreover, metals such as iron and copper interact with free radicals which contribute to the propagation of the lipid peroxidation chain reaction. It is evident then that a single initiating event, caused by a prooxidant, may cascade into a widespread chain reaction that produces many deleterious products in concentrations greater than that of the initiator. This is exemplified by the fact that thousands of molecules may be destroyed by a lipid peroxidation chain reaction initiated by a single radical. It is imperative that in order to prevent this vicious chain reaction, the O_2 radical cascade to O_2 and H_2O_2 must be attenuated, and the peroxides converted to innocuous metabolites. All aerobic organisms therefore possess elaborate defense mechanisms to prevent the formation of toxic forms of oxygen and to remove any peroxides formed.

1.5 Oxidative stress and human disease

Reactive oxygen species (ROS) such as superoxide anions, hydrogen peroxide, and hydroxyl, nitric oxide and peroxy nitrite radicals, play an important role in the pathogenesis of various diseases. The constant attack by oxyradicals and reactive oxygen species (ROS) contributes to both the initiation and the progression of many major diseases. The oxidation of lipid, DNA, proteins, carbohydrates and other biological molecules by toxic ROS may cause mutation and damage to cells or tissues. The last decade has yielded considerable evidence that implicates oxidative stress as a factor in the etiology and progression of a spectrum of diseases, which include atherosclerosis, cancer, eye disorders, Parkinson

disease, diabetes, gastric ulcers, liver diseases etc. The mechanism may differ in specific diseases, but generation of ROS is found in all cases [48].

1.6 Antioxidant defense system

All aerobic forms of life maintain elaborate defense systems known as antioxidant systems to protect the body against free radical damage. The body needs to strike the right balance between the number of free radicals generated and the defense and repair mechanism available. The current view of cellular oxidant defenses can be categorized into primary and secondary defense systems [49, 50]. The primary defenses consists of the broadly studied antioxidant compounds, such as α-tocopherol, ascorbic acid, β-carotene and uric acid, along with variety of antioxidant enzymes, where superoxide dimutase (SOD), catalase (CAT) and glutathione peroxidase (GSH-Px) are notable examples.

Secondary defenses are predominantly a series of enzyme systems that act to repair or eliminate molecules or cell components that were damaged by oxidants or free radical reactions, which escape the primary antioxidant defense [51].

1.7 Role of medicinal plants as antioxidants

The widespread use of traditional herbs and medicinal plants has been traced to the occurrence of natural products with medicinal properties. In recent years, the traditional medicine, the world has revalued by an extensive activity of research on different plant species and their therapeutic principles. Various medicinal properties have been ascribed to natural herbs and medicinal plants constitute one of the main source of new pharmaceuticals and healthcare products. Many studies have been performed to identify antioxidant compounds with pharmacological activity with limited toxicity. A whole range of plant derived dietary supplements, phytochemicals and pro-vitamins that assist in maintaining good health and combating disease are now being described as functional foods, nutriceuticals and nutraceuticals [52].

Potential sources of antioxidant compounds have been searched in many types of plant materials such as fruits, seeds and leaves etc. As plants produce a lot of antioxidants to

control the oxidative stress caused by sunbeams and oxygen, they can represent a source of new compounds with antioxidant activity. It has been observed that phytochemicals like tannic acid, flavonoids, tocopherol, curcumin, ascorbate, carotenoids, polyphenols, etc. were reported to have potent antioxidant properties [53].

1.8 Oxidative stress and diabetes

The sources of oxidative stress in diabetes are nonenzymatic, enzymatic and mitochondrial pathways [54]. Nonenzymatic sources of oxidative stress originate from the oxidative biochemistry of glucose. Hyperglycemia can directly cause increased ROS generation. Glucose can undergo autoxidation and generate $^{\cdot}OH$ radicals. Glucose reacts with proteins in nonenzymatic pathway. ROS is generated at multiple steps during this process. In hyperglycemia, there is enhanced metabolism of glucose through the polyol (sorbitol) pathway, which results in enhanced production of $^{\cdot}O_2^-$. Enzymatic sources of oxidative stress in diabetes include NOS, NAD(P)H oxidase and xanthine oxidase. All isoforms of NOS require five cofactors such as flavin adenine dinucleotide (FAD), flavin mononucleotide (FMN), heme, BH_4 and Ca^{2+}-calmodulin. If NOS lacks one of its cofactors, NOS may produce $^{\cdot}O_2^-$ instead of $^{\cdot}NO$ and this is referred as the uncoupled state of NOS. NAD(P)H oxidase is a membrane associated enzyme that consists of five subunits and is a major source of $^{\cdot}O_2^-$ production. The mitochondrial respiratory chain is another source of nonenzymatic generation of reactive species. During the oxidative phosphorylation process, electrons are transferred from electron carriers NADH and $FADH_2$ through four complexes in the inner mitochondrial membrane to oxygen generating ATP in the process. The $^{\cdot}O_2^-$ is immediately eliminated by natural defense mechanism in normal conditions. The hyperglycemia induced generation of $^{\cdot}O_2^-$ at the mitochondrial level is the initial trigger of oxidative stress in diabetes. When endothelial cells are exposed to hyperglycemia at the levels relevant to clinical diabetes, there is increased generation of ROS and especially $^{\cdot}O_2^-$, precedes with the development of diabetic complications.

Reactive species can be eliminated by a number of enzymatic and nonenzymatic antioxidant mechanisms [55]. The SOD immediately converts $^{\cdot}O_2^-$ to H_2O_2, which is then detoxified to water either by catalase in the lysosome or by glutathione peroxidase in the mitochondria.

The gluatathione reductase acts as hydrogen donor during the elimination of H_2O_2. Nonenzymatic antioxidants include vitamin A, C and E, glutathione, α-lipoic acid, carotenoid, trace elements like copper, zinc and selenium, coenzyme Q_{10} (CoQ_{10}) and cofactors like folic acid, uric acid, albumin and vitamin B_1, B_2, B_6 and B_{12}. Glutathione (GSH) acts as a direct scavenger and co-substrate for GSH peroxidase. Vitamin E is a fat soluble vitamin that prevents lipid peroxidation. CoQ_{10} is a lipid soluble antioxidant, in higher concentrations it scavenges $^{\cdot}O_2^-$ and improves endothelial dysfunction in diabetes. Vitamin C increases NO production in endothelial cells by stabilizing NOS cofactor BH_4. α-Lipoic acid is reduced to dihydrolipoate. Dihydrolipoate is able to regenerate antioxidants such as vitamin C, vitamin E and reduced glutathione through redox cycling.

Free radicals and other reactive species play an important role in many human diseases. Plants have long been regarded as having considerable health benefits, due to their main antioxidant compounds [56]. In living system, free radicals are generated as part of the body's normal metabolic process. The free radical chain reactions are usually produced in the mitochondrial respiratory chain, liver mixed function oxidases, through xanthine oxidase activity, atmospheric pollutants and from transitional metal catalysts, drugs and xenobiotics. Oxygen free radical can initiate peroxidation of lipids, which in turn stimulates glycation of proteins, inactivation of enzymes and alteration in the structure and function of collagen basement and other membranes and play a role in the long term complication of diabetes.

Diabetes is a risk factor for cardiovascular disease. The microvascular complications of diabetes include nephropathy and retinopathy, macrovascular complications are coronary artery disease, cerebrovascular disease and peripheral vascular disease are the leading cause of death in the diabetes [57]. The control of blood glucose is effective in reducing the clinical complications. The oxidative stress mediated mainly by hyperglycemia induced generation of free radicals. The antioxidants treatments are effective in reducing diabetic complications. Several clinical trials investigated the effect of antioxidant vitamin E on the prevention of diabetic complications. These clinical trials are failed to demonstrate relevant clinical benefits of this antioxidant on cardiovascular disease. The negative results of the clinical

trials with antioxidants lead to focus on mechanism of oxidative stress in diabetes to develop antioxidant therapy.

1.9 Diabetes mellitus

Diabetes mellitus is a chronic disease of metabolic disorder caused by deficiency in production of insulin by the β-cells of pancreas. This results in increased concentration of blood glucose. This uncontrolled hyperglycemia after long duration leads to retinopathy, neuropathy, nephropathy, cardiovascular problems and damage to blood vessels [58, 59]. The blood glucose level in the human body is balanced by insulin and glucagon. The normal blood sugar of human body should be between 70 mg/dl to 110 mg/dl at fasting state and below 140 mg/dl at two hours after eating. If blood glucose level is less than 70 mg/dl is termed as hypoglycemia and more than 110 mg/dl is termed as hyperglycemia.

Insulin deficiency is the major cause in Type-1 diabetes, in which pancreas stop producing insulin. In Type-2 diabetes, the cause may be inefficient utilization of glucose by human body cells [60, 61]. The Type-3 diabetes is termed as Gestational diabetes and it is due to development of insulin resistance. Gestational diabetes affects the mother and the baby. According to W.H.O estimate, by 2025, a total of 300 million of the worldwide population will be affected by diabetes and W.H.O recommended to include traditional medicines in primary healthcare centers of third world countries, where 80% of the population depend on traditional medicines. The traditional medicines constitute the plant products and plant derived products. The plant products, plant derived active principles and synthetic drugs are used in the treatment of Type-2 diabetes.

The pathophysiology of all types of diabetes is related to the hormone insulin, which is secreted by the beta cells of the pancreas [62, 63]. In a healthy person, insulin is produced in response to the increased level of glucose in the bloodstream, and its major role is to control glucose concentration in the blood. What insulin does is, allowing the body cells and tissues to use glucose as a main energy source. Also, this hormone is responsible for conversion of glucose to glycogen for storage in the muscles and liver cells. This way, sugar level is maintained at a near stable amount.

In a diabetic person, there is an abnormal metabolism of insulin hormone. The actual reason for this malfunction differs according to the type of diabetes. Whatever the cause is, the body cells and tissues do not make use of glucose from the blood, resulting in elevated blood glucose (a typical symptom of diabetes called hyperglycemia). This condition is also exacerbated by the conversion of stored glycogen to glucose, i.e., increased hepatic glucose production. Over a period of time, high glucose level in the bloodstream can lead to severe complications, such as eye disorders, cardiovascular diseases, kidney damage, and nerve problems.

In Type 1 diabetes, the pancreas cannot synthesize enough amounts of insulin as required by the body. The pathophysiology of Type 1 diabetes mellitus suggests that it is an autoimmune disease, wherein the body's own immune system generates secretion of substances that attack the beta cells of the pancreas. Consequently, the pancreas secretes little or no insulin. Type 1 diabetes is more common among children and young adults (around 20 years). Since it is common among young individuals and insulin hormone is used for treatment, Type 1 diabetes is also referred to as Juvenile Diabetes or Insulin Dependent Diabetes Mellitus (IDDM).

In case of Type 2 diabetes mellitus, the insulin hormone secreted by the beta cells is normal or slightly lower than the ideal amount. However, the body cells are not responding to insulin as they do in a healthy person. Since the body cells and tissues are resistant to insulin, they do not absorb glucose, instead it remains in the bloodstream. Thus, the Type 2 diabetes is also characterized by elevated blood sugar. It is commonly manifested by middle-aged adults (above 40 years). As insulin is not necessary for treatment of Type 2 diabetes, it is known as Non-insulin Dependent Diabetes Mellitus (NIIDM).

The third type of diabetes is called Gestational diabetes. As the term clearly suggests, it is exhibited by pregnant women. Over here, high level of blood glucose is caused by hormonal fluctuations during pregnancy. Usually, the sugar concentration returns to normal after the baby is born. However, there are also instances, in which it remains high even after childbirth. This is an indication for increased risks of developing diabetes in the near future.

As already mentioned, the symptoms and effects of all the three forms of diabetes are similar [64, 65]. The noticeable symptoms include increased thirst (polydipsia), increased urination (polyuria), and increased appetite (polyphagia). Other diabetes signs and symptoms include excessive fatigue, presence of sugar in the urine (glycosuria), body irritation, unexplained weight loss, and dehydration. Elevated blood sugar and glycosuria are interrelated; when sugar amount in the blood is abnormally high, the reabsorption by proximal convoluted tubule is reduced, thereby retaining some glucose in the urine.

2. SCOPE, OBJECTIVES AND PLAN OF WORK

2.1 SCOPE OF THE WORK

Actiniopteris radiata is a desert fern belong to family Adiantaceae (Pteridaceae). It is a tiny terrestrial fern, found throughout India and also in Burma, Sri Lanka, Afghanistan, Persia, Arabia, Yemen, South Eastern Egypt, Tropical Africa, Australia and Madagascar. It is of limited distribution, and in areas where it occurs, is restricted to depleted walls and rocky crevices of steep slops of exposed hilly areas, up to the altitude of 1200 m. The term Actiniopteris has its origin from the Greek aktis (ray) and pteris (fern); refers to the radiating leaf segments. Its vernacular names include Mayursikha : Sanskrit; Mapursika :Bombay and Peacock's tail :English.

Adiantaceae family has cosmopolitan ferns, about 17 species occur in India, most of which possess medicinal properties. The ferns are primarily plants of lower elevation, growing upto 600 m above sea level, but a few survive at higher elevations also. The plants of this family are reported to contain kaemferol, quercetol, luteol, adiantone, isoadiantone, fernene, β-sitosterol and quercetin. The plants are used as hypoglycaemic, hair tonic, in skin diseases, leprosy and fever. An ointment prepared from fern is used as hair tonic. The decoction of the plant is used to cure cough and cold.

The ethnomedical uses of this plant are anthelmintic, haemostatic, antileprotic, used in dysentery, diabetes, skin diseases and fever. The reported biological activities of this plant are analgesic, antihistaminic, antimicrobial, antifungal and antifertility activity. The plant contain rutin, hentricontane, hentricontanol, β-sitosterol, β-sitosterol palmitate, unidentified glucoside, glucose and fructose. The reported phytochemical work is less.

2.2 OBJECTIVES OF THE WORK

1. To select the plants based on their ethnomedical uses and preparation of their extracts.
2. To screen the extracts for *in vitro* antioxidant activity.

3. To screen the extracts for *in vitro* antidiabetic activity.
4. To screen the plant extract for *in vivo* antidiabetic activity
5. To isolate the chemical constituents from the plant extract and structure elucidation.

2.3 PLAN OF WORK

- Selection of the plant, authentication, the whole plant to be dried at room temperature.
- The coarsely powdered plant to be extracted with different solvents of increasing polarity.
- Qualitative phytochemical analysis and quantitative phytochemical estimation of extracts.
- Column chromatography of ethyl acetate extract.
- Fractionating the ethyl acetate extract.
- Evaluation of *in vitro* anti-diabetic activity by alpha glucosidase inhibition activity.
- Quantitative and qualitative estimation of ethyl acetate extract and fractions by HPTLC.
- Characterisation of isolated compounds by melting point, UV, IR, NMR, and mass spectrums.
- Evaluation of the extracts for *in vitro* antioxidant activity.
- Evaluation of the extracts for *in vivo* anti-diabetic activity.

3. PLANT PROFILE AND REVIEW OF LITERATURE

3.1 *Actiniopteris radiata*

Family : Polypodiaceae

Vernacular Names : English : Peacock's tail
Hindi : Mayursikha
Kannada : Mayurasikha
Malayal : Mayurasikha
Sanskrit : Mayursikha
Tamil : Mayilatumsikhai
Telagu : Mayurasikha

Distribution:

It is found throughout India.

Figure 1: Structure of *Actiniopteris radiata*

Description:

A herbaceous miniature palm like fern upto 25 cm hight with densely tufted stipe. Fronds fan like with numerous dichotomous segments which are rush like in texture, veins few, subparallel with distinct midrib, segments of fertile frond longer than those of the barren one, sori linear, elongate, submarginal.

Ethnomedical information:

The plant is bitter, astringent, anthelmintic, haemostatic, antileprotic and febrifuge. It is useful in vitiated conditions of kapha and pitta, diarrhea, dysentery, helminthiasis, haemoptysis, leprosy, skin diseases, diabetes and fever [66].

Chemical Constituents:

The plant contains rutin, hentriacontane, hentricontanol, β-sitosterol, β-sitosterol palmitate, β-sitosterol-D-(+)-glycoside, an unidentified glycoside, glucose and fructose.

3.2 Phytochemical investigation and biological activity

The research papers have been collected for phytochemical investigation, in-vivo anti-diabetic screening and in-vitro antioxidant activity for the selected plant *Actiniopteris radiata* and related plants. Bambie, et al., have reported the gametophytic and sporophytic generations of this plant [67]. *Actiniopteris radiata* is one of the apogamously developed xerophytic ferns of Actiniopteridaceae. It has been worked out in detail regarding its anatomy and morphology. The development of its gametophytes has also been studied up to the 8-celled stage after which they did not grow on artificial medium. The spores are trilete with slightly convex sides and rounded corners. The leasurae are long, crassimarginate with undulate surface. Spores bear large, irregularly closely-set verucae like ridge with wavy margins. They are yellowish to dark brown when mature. The average dimensions of the spores are 49.39 × 54.83 µ. On germination the spore forms a densely chlorophyllous germ filament composed of 3 to 8 short barrel shaped cells. The gametophytic and sporophytic generations of *actiniopteris radiata* clearly indicate that this plant has adapted itself very well to the xeric environment where it usually grows.

Bambie, et al., have reported the preliminary study of the chemical constitution of the plant *Actiniopteris radiata* [68]. Dried stems and leaves of the plant (500 g) were extracted with petroleum ether and ethanol respectively. The petroleum ether extract was concentrated under reduced pressure to a green solid mass (10 g). It was put over an alumina column and eluted successively with petroleum ether (40-60^{o}C), petroleum ether: benzene (4:1), benzene and benzene : chloroform (1:1). The first few fractions from petroleum ether on evaporation gave a 20 mg of hentricontane. The fractions after elution with petroleum ether : benzene (4:1) gave a 100 mg of β-sitosterol palmitate. The elutes from pure benzene on evaporation gave 100 mg of hentricontol. The alcoholic extract of the plant was concentrated to one-tenth of its original volume and was kept at 0^{o}C for few days. A yellow crystalline substance was accumulated at the bottom. This on repeated crystallization from methanol gave yellow crystalline substance mp 190^{o}C. It failed to give the test for steroids and flavanoids. It gave positive response for Molisch's test and blood red coloration with conc.H_2SO_4 indicates the glycosidic nature of the compound.

Taneja, et al., have reported the isolation of compounds from petroleum ether and ethanol extract and reported the presence of 3-hydroxy flavones in the ethanol extract [69].

Actiniopteris radiata was evaluated for analgesic activity using ethanolic and aqueous extract by acetic acid induced writhing method and tail flick method [70]. Albino mice weigh 20-25 g were divided into four groups consisting of six animals. Group one served as negative control, group second served as positive control (Pentazocine 5 mg/kg b.w ip), group third received aqueous extract (300 mg/kg b.w ip) and group fourth received ethanolic extract (300 mg/kg b.w ip) of *Actiniopteris radiata*. The writhing movements were observed and counted for a period of 15 minutes after acetic acid administration. The mean writhing scores in control, extracts and pentazocine treated groups were calculated. All animals were individually exposed to tail flick apparatus maintained at 55^{o}C. The tail withdrawn from the heat is taken as the end point. Cut off period of 10-12 sec is observed to prevent damage to tail. The reaction time was noted from 0, 30, 60, 90, 120 and 180 minutes time interval. The aqueous and ethanolic extracts shows significant analgesic activity in writhing method.

Whereas intraperitoneal administration of the aqueous and ethanolic extracts of *Actiniopteris radiata* showed non significant change in the tail flick latency till 120 minutes.

Actiniopteris radiata was tested for in-vitro antihistaminic and anticholinergic activity [71]. Male wistar rats were sacrificed and a segment from ileum was dissected from the terminal ileum and mounted in organ bath containing tyrode solution. A dose response curve for histamine and acetylcholine was recorded in the following groups. Group 1- control (Histamine and Ach), group 2- vehicle, group 3- test extract (2 mg/ml), group 4- test extract (4 mg/ml), group 5 – test extract (10 mg/ml). The ethanolic extract of *Actiniopteris radiata* shown significant antihistaminic and anticholinergic activity.

4. MATERIALS AND METHODS

4.1 Plant Material

The whole plant of *Actiniopteris radiata* was collected from Nilgiri district, Tamil Nadu, India, in November 2007. The plant was identified by Dr. S. Rajan, Field Botanist, Survey of Medicinal Plants and Collection Unit, Emerald, Nilgiri (Voucher No: 135). A voucher specimen was deposited at Survey of Medicinal Plants and Collection Unit, Emerald, Nilgiri.

4.2 Materials

4.2.1 Instruments

Melting points were determined using a Lab India melting point apparatus. UV-Visible spectrums were recorded using a Shimadzu UV-1700. IR spectrums were recorded on a Shimadzu FTIR-8400s. ^{1}H (500 MHz) and ^{13}C (100 MHz) spectrums were recorded on a BRUKER AV-400. EIMS was recorded by GC-MS on a P-POS/TOP MICRO, HITACHI. ESIMS spectrums were recorded on a HCT-Ultra PTM discovery, BRUKER. ELISA reader data recorded on a BIO - RAD 550.

4.2.2 Chemicals

2, 2 –diphenyl -1- picryl hydrazyl (DPPH) and 2, 2^{1}- azino-bis (3-ethylbenz-thiazoline-6-sulfonic acid) diammonium salt (ABTS) were procured from Sigma-Aldrich, California, USA. Rutin and *p*-nitroso dimethyl aniline (*p*-NDA) were procured from Acros Organics, New Jersy, USA. Naphthyl ethylene diamine dihydrochloride (NEDD) was procured from Roch – Light Ltd, Suffolk, UK. Nitro blue tetrazolium (NBT) was procured from S.D Fine Chem Ltd, Biosar, India. Glibenclamide was procured from Inga labs Ltd, Mumbai, India. Streptozotocin was procured from Hi media, Mumbai, India. All the other chemicals used were of analytical grade.

4.3 Preparation of the plant extract

The plant was dried under shade for 7 days. The coarsely powdered plant material (500g) was packed in soxhlet apparatus. The packed plant material was extracted successively with petroleum ether, chloroform, ethyl acetate and ethanol for 18-20 hrs. These extracts were filtered and dried under vacuum.

4.4 Preliminary phytochemical analysis of successive extracts of *Actiniopteris radiata*

The qualitative chemical tests were carried out for successive extracts of *Actiniopteris radiata* to identify the chemical constituents.

- **Test for alkaloids**
  - Mayer test
  - Dragendroff's test
  - Wagner test
  - Hager test
- **Test for saponins**
  - Foam test
- **Test for carbohydrates**
  - Molisch test
  - Benedict test
- **Test for glycosides**
  - Borntrager test
  - Test for reducing sugar
- **Test for steroids**
  - Libermann Buchard test
- **Test for fatty acids**
  - Saponification test
- **Test for flavanoids**
  - Ferric chloride test

4.5 Physicochemical analysis

4.5.1 Ash value

Total ash

The powdered plant (3 g) was accurately weighed and spread in a silica crucible which was previously ignited and weighed. The crucible was incinerated at a temperature not exceeding 450°C to make the powder free from carbon. The procedure was repeated to get a constant weight. The percentage of a total ash was calculated with reference to the dry weight of the powdered plant [72].

Acid insoluble ash

The acid insoluble ash was determined from the total ash. The total ash was boiled with 25 ml of 2 N HCl for 5 min. The insoluble ash was collected on an ash less filter paper and washed with hot water. The insoluble ash was transferred to pre-weighed silica crucible, ignited, cooled and weighed. The procedure was repeated to get a constant weight. The percentage of an acid insoluble ash was calculated with reference the dry weight of the powdered plant.

Water soluble ash

The water soluble ash was determined from the total ash. The total ash was boiled with 25 ml. of distilled water for 5 min. The insoluble ash was collected on an ash less filter paper and washed with hot water. The insoluble ash was transferred to pre-weighed silica crucible, ignited, cooled and weighed. The procedure was repeated to get a constant weight. The percentage of water soluble ash was calculated with reference to the dry weight of the powdered plant.

4.5.2 Extractive value

Extractive value determines the amount of active constituents in a given amount of medicinal plant material when extracted with solvent [73].

Alcohol soluble extractive value

The powdered plant (3 g) was macerated with alcohol (50 ml) in stoppered flask for 24 h and filtered. The filtrate was evaporated at 105°C to get a residue. The dry weight of the residue

was taken and percentage of alcohol soluble extractive value was calculated from the dry weight of the powder.

Water soluble extractive value

The powdered plant (3 g) was macerated with water (50 ml) in stoppered flask for 24 h and filtered. The filtrate was evaporated at 105˚C to get a residue. The dry weight of the residue was taken and percentage alcohol soluble extractive value was calculated from the dry weight of the powder.

Moisture content

Moisture content was determined by subjecting the plant material at 105˚C to constant weight and total loss of weight was calculated. The moisture content of the plant material was determined by using Sartorius electronic moisture balance, a process of drying and its simultaneous weight recording up to the point of constant weight.

4.6 Isolation of compounds and characterisation

4.6.1 Column Chromatography

Isolation of compounds from extracts was done by selection of silica gel (60-120 mesh size) column chromatography. The column was prepared by wet packing method. The mobile phase was allowed to flow down through the column. The plant extract was dried to free flow powder, packed in a column chromatography. The solvents were allowed to flow down in the order of increasing polarity [74, 75, 76].

4.6.2 Column chromatography of ethyl acetate extract

The ethyl acetate extract showed significant activity in the preliminary studies carried out and hence it was selected for further fractionation and isolation. Fractionation was carried out using silica gel column. The column was packed by wet packing method using petroleum ether as solvent. The extract dried under vacuum was found to be 16.0 g. It was packed in a column chromatography with a silica gel 60-120 mesh size as adsorbent (300.0 g). The mobile phase was allowed to flow through the column in the increasing order of polarity [77, 78, 79]. The fractions were collected as follows.

Thin layer chromatography was performed for all collected fractions and the fractions showing similar chromatograms were combined. The purification was done for major fractions by re-column. The fraction 13 was evaporated to yield 380 mg of yellow residue. It was purified by column chromatography to yield 45 mg of compound 1. The fraction 15 was evaporated to yield 120 mg of yellow residue. It was purified by column chromatography to yield 30 mg of compound 2 [80, 81, 82].

Isolation of compounds in ethyl acetate extract was performed as given below.

Fractions No	**Solvent system**	**Observation**
1	Pet. ether :100	Green residue
2	Pet. ether : $CHCl_3$:95 :5	Green residue
3	Pet. ether : $CHCl_3$:90 :10	Brown residue
4	Pet. ether : $CHCl_3$: 85:15	Brown residue
5	Pet. ether : $CHCl_3$: 80:20	Yellow residue
6	$CHCl_3$: 100	Yellow residue
7	$CHCl_3$: Ethyl acetate : 95 : 5	Yellow residue
8	$CHCl_3$: Ethyl acetate : 90 : 10	Yellow residue
9	$CHCl_3$: Ethyl acetate : 85 : 15	Yellow residue
10	$CHCl_3$: Ethyl acetate : 80 : 20	Yellow residue
11	Ethyl acetate : 100	Yellow residue
12	Ethyl acetate : Methanol : 95 : 5	Yellow residue
13	Ethyl acetate : Methanol : 90 : 10	Yellow residue
14	Ethyl acetate : Methanol : 85 : 15	Yellow residue
15	Ethyl acetate : Methanol : 80 : 20	Yellow residue
16	Methanol : 100	No residue

Fraction 13 (380 mg) was packed in column chromatography (silica gel 60-120 mesh size, 30 g). The solvents were allowed to flow in the order of increasing polarity. Twelve fractions were collected. Fraction 11 yielded 45 mg of compound 1.

Isolation of compound 1 from fraction 13

Fractions No	**Solvent system**	**Observation**
1	Pet. ether ; 10	No residue
2	Pet. ether: $CHCl_3$;9:1	Yellow residue
3	Pet. ether : $CHCl_3$;8:2	Yellow residue
4	Pet. ether : $CHCl_3$;7:3	No residue
5	$CHCl_3$;10	Yellow residue
6	$CHCl_3$: Ethyl acetate ; 9:1	Yellow residue
7	$CHCl_3$: Ethyl acetate ; 8: 2	Yellow residue
8	$CHCl_3$: Ethyl acetate ; 7: 3	No residue
9	Ethyl acetate ; 10	Yellow residue
10	Ethyl acetate : Methanol ; 9:1	Yellow residue
11	Ethyl acetate : Methanol ; 8:2	Yellow residue
12	Ethyl acetate : Methanol ; 7:3	No residue

Fraction 15 (120 mg) was packed in column chromatography (silica gel 60-120 mesh size, 30 g). The solvents were allowed to flow in the order of increasing polarity. Thirteen fractions were collected. Fraction 13 yielded 30 mg of compound 2.

Isolation of compound 2 from fraction 15

Fractions No	Solvent system	Observation
1	Pet. ether ; 10	No residue
2	Pet. ether: $CHCl_3$;9:1	Yellow residue
3	Pet. ether : $CHCl_3$;8:2	Yellow residue
4	Pet. ether : $CHCl_3$;7:3	No residue
5	$CHCl_3$; 10	Yellow residue
6	$CHCl_3$: Ethyl acetate ; 9:1	Yellow residue
7	$CHCl_3$: Ethyl acetate ; 8: 2	Yellow residue
8	$CHCl_3$: Ethyl acetate ; 7: 3	Yellow residue
9	Ethyl acetate ; 10	No residue
10	Ethyl acetate : Methanol ; 9:1	Yellow residue
11	Ethyl acetate : Methanol ; 8:2	Yellow residue
12	Ethyl acetate : Methanol ; 7:3	No residue
13	Methanol ; 10	Yellow residue

4.7 QUANTITATIVE PHYTOCHEMICAL SCREENING

4.7.1 Estimation of total phenolic content

Total phenolic content was determined by using the Folin-ciocalteu method. This test is based on the oxidation of phenolic groups with phosphor molybdic and phosphor tungstic acids. After oxidation a green-blue complex formed which was measured at 750 nm [83].

Chemicals and reagents

i. Folin-ciocalteu reagent: Folin-ciocalteu reagent was diluted (1:10) with distilled water and used.
ii. Sodium carbonate: (0.7 M) 7.420 g of sodium carbonate was dissolve in 100 ml of distilled water.
iii. Methanol
iv. Preparation of test Solutions: 5 mg each of the extract and its fraction were separately dissolved in 5 ml of methanol to get 1 mg/ml solution.
v. Preparation of Standard Solutions: Gallic acid monohydrate (5 mg) was dissolved in 50 ml distilled water to get (100 µg/ml). It was serially diluted with distilled water to obtain lower dilutions of 80, 60, 40 and 20 µg/ml.

Procedure

The test and standard solutions (1 ml) were separately mixed with distilled water (5 ml), ethanol (1 ml), folin-ciocalteu reagent (0.5 ml) and sodium carbonate (1 ml). The reaction mixture was mixed thoroughly. After 2 h the absorbance was measured at 750 nm. Using the gallic acid standard curve the total phenolic contents of the sample were calculated. The total phenolic content was expressed in terms of gram percentage (g %).

Estimation of total flavonoid content

Total flavonoid content is determined by aluminum chloride method. The principle of this method is that aluminum chloride forms acid stable complexes with the C-4 keto group and either the C-3 or C-5 hydroxyl groups of flavones and flavonols. In addition, aluminum

chloride forms acid labile complexes with the ortho-dihydroxyl groups in the A or B ring of flavonoids. The concentration of these complexes was measured at 415 nm [84].

Chemicals and reagents

i. Aluminium Chloride 10% w/w: 10 g of aluminium chloride was dissolved in 100 ml of distilled water.
ii. Potassium acetate (1 M): 98.10 g of potassium acetate was dissolved in 1 liter of distilled water.
iii. Distilled Methanol.
iv. Preparation of test Solutions: 5 mg each of the extract and its fraction were separately dissolved in 5 ml of methanol to get (1 mg/ml) solution.
v. Preparation of Standard Solutions: Rutin monohydrates (5 mg) was dissolved in 50 ml methanol to get 100 µg/ml. The primary stock was serially diluted with methanol to obtain lower dilutions of 80, 60, 40 and 20 µg/ml.

Procedure

The test and standard solutions (0.5 ml) were separately mixed with distilled water (2.8 ml), methanol (1.5 ml), aluminium chloride (0.1 ml) and potassium acetate (0.1 ml) and incubated at room temperature for 20 minutes. The absorbance of the reaction mixture was measured at 415 nm. Using the rutin standard curve, the total flavonoid content of samples calculated. The total flavonoid content was expressed in terms of gram percentage (g %).

QUANTITATIVE AND QUALITATIVE ANALYSIS OF EXTRACT AND FRACTION

The quantitative and qualitative estimation of the extract and fraction was done by HPTLC method [85].

4.8.1 Qualitative estimation of ethyl acetate extract, fraction and sample preparation for HPTLC

100 mg of Extract and Fraction were dissolved in 10 ml of methanol and sonicated for 30 min to get 10mg/ml solution.

Sample preparation : 1mg/ml solution in methanol
Stationary phase : Precoated Silica gel F 254 Plates (MERCK)

Mobile phase : Chloroform: Methanol
Saturation : 30 mins
Development chamber : CAMAG twin trough development chamber
Applicator : CAMAG Linomat IV applicator
Scanner : CAMAG Scanner III CATS (4.06), Switzerland
Mode of scanning : Absorption (deuterium)
Detection wavelength : 254-366 nm
Volume applied (samples) : 5µl of above prepared crude extract were applied.

Quantitative estimation of ethyl acetate extract and fraction

Standard Preparation

10 mg of Quercetin, rutin, gallic acid, ursollic acid, piperin and catechins were dissolved in 10 ml of methanol seperately to get 1mg/ml solution.

Sample preparation
100 mg of extract/fraction were dissolved in 10 ml of methanol and sonicated for 30min to get 10mg/ml solution.
Stationary phase : Precoated silica gel TLC plates GF60

	Mobile phase	Ratio	Wave length (nm)	Volume applied			
				Standard		Sample (µl)	
				Vol. (µl)	Conc. (ng)	Vol. (µl)	Conc (ng)
Quercetin	Ethyl acetate:formic acid:glacial acetic acid:water	10:1.1: 1.1:2.6	200 - 366	1 2 4 6 8	1000 2000 4000 6000 8000	20	
Rutin	Ethyl acetate:formic acid:glacial acetic acid:water	10:1.1: 1.1:2.6	200- 366	1 2 4 6 8	1000 2000 4000 6000 8000	20	
Gallic acid	Toulene:acetone: formic acid	7:5:1	200- 366	1 2 4 6 8	1000 2000 4000 6000 8000	20	
Ursollic acid	Chloroform:Methanol	9:1	200- 366	1 2 4 6 8	1000 2000 4000 6000 8000	20	

Piperine	Chloroform:Methanol	9.25:0.75	200- 366	1 2 4 6 8	1000 2000 4000 6000 8000	20	
catechins	Toulene:ethyl acetate:formic acid	4:5:1	200- 366	1 2 4 6 8	1000 2000 4000 6000 8000	20	

Linearity detector response (calibration by linear regression technique)

Linearity of detector response for all possible markers was performed using 1mg/ml-working solution, five different concentrations µg (10, 20, 40, 60 and 80) were applied on the HPTLC plates. The linearity was determined according to their peak area and peak height.

Spectral matching (Densitometric scan)

The spectras of standard and sample were matched to confirm the components by matching the standard R_f value and spectral scanning was carried out at 200-700 nm.

4.9 *In-Vitro* antioxidant activity

Free radicals are continuously produced by the body's normal use of oxygen. The balance between the amount of free radicals generated in the body and antioxidants to scavenge them to protect the body against hyperglycemic related retinopathy, hypertension, cancer, diabetes mellitus, cardiac disorders, alzheimer's disease and nephropathy. These disorders are primarily due to imbalance between pro-oxidants and anti-oxidants. The natural products like plants and plant products are correcting the imbalance [86]. There are many methods for evaluation of antioxidant activity. The *in vitro* methods are based on inhibition of free radicals. Samples are added to a free radical generating system and the inhibition of the free radical activity is measured. This inhibition is related to antioxidant activity of the sample. Methods vary greatly as to the generated radical, the reproducibility of the generation process and the end point that is used for the determination.

Even though *in vitro* methods provides a useful indication of antioxidant activities, data obtained from *in vitro* methods are difficult to apply to biological systems and do not

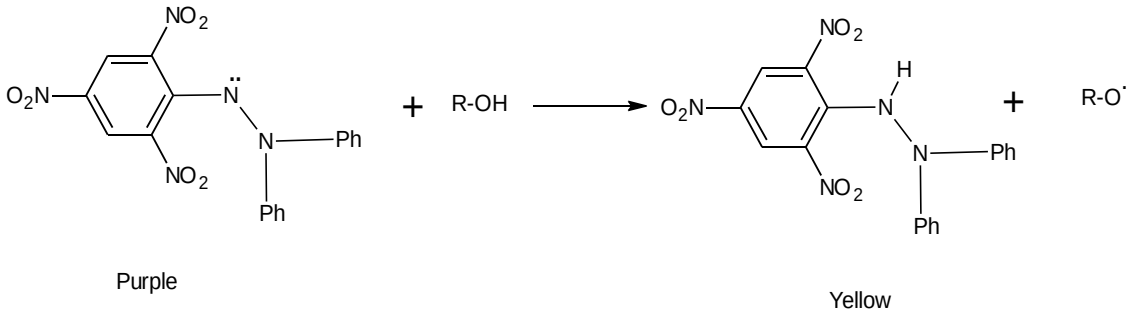
necessarily predict a similar *in vivo* antioxidant activity. All the methods developed have strengths and limitations and hence a single measurement of antioxidant capacity usually is not sufficient. A number of different methods may be necessary to adequately assess *in vitro* antioxidant activity of a extracts. In the present study all the extracts were tested for *in vitro* antioxidant activity using several standard methods. The absorbance was measured spectrophotometrically against the corresponding blank solution. The percentage inhibition was calculated by using the formula,

$$\text{Percentage inhibition} = \frac{OD_{control} - OD_{sample}}{OD_{control}} \times 100$$

The quality of antioxidants in the extracts and fractions were determined by the IC_{50} values. A low IC_{50} value indicates strong antioxidant activity in the extracts or fractions. The evaluation of antioxidant activity was performed by following methods.

4.9.1 DPPH radical scavenging activity

The DPPH free radical is reduced to a corresponding hydrazine when it reacts with hydrogen donors. The DPPH radical is purple in colour and upon reaction with a hydrogen donor changes to yellow in colour. It is a discoloration assay, which is evaluated by the addition of antioxidant to a DPPH solution in ethanol or methanol and the decrease in absorbance was measured at 490 nm [87, 88, 89].



Purple

Yellow

Reagents

DPPH solution (100μM): Accurately 22 mg of DPPH was weighed and dissolved in 100 ml of methanol. From this stock solution, 18 ml was diluted to 100 ml with methanol to obtain 100 μM DPPH solution.

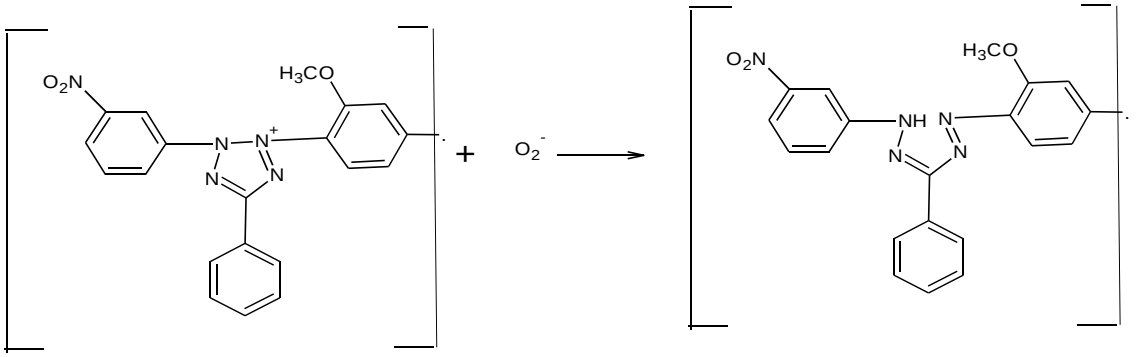
Preparation of extract solutions: Accurately 21 mg of each of the extracts were weighed and dissolved in 1 ml of freshly distilled DMSO separately to obtain solutions of 21 mg/ml concentration. These solutions were serially diluted separately to obtain lower concentrations.

Preparation of standard solutions: Accurately 10 mg each of ascorbic acid and rutin were weighed and dissolved in 0.95 ml of freshly distilled DMSO separately to obtain 10.5 mg/ml concentration. These solutions were serially diluted with DMSO to get lower concentrations.

Procedure: The assay was carried out in 96 well microtitre plate. To 200 µl of DPPH solution, 10 µl of each of the extract or standard solution was added separately in wells of the microtitre plate. The plates were incubated at 37°C for 30 min and the absorbance of each solution was measured at 490 nm, using ELISA reader.

4.9.2 Superoxide radical scavenging activity by alkaline DMSO method

In alkaline DMSO method, superoxide radical is generated by the addition of sodium hydroxide to air saturated dimethyl sulfoxide (DMSO). The generated superoxide remains stable in solution, which reduces nitro blue tetrazolium into formazan dye at room temperature and this can be measured at 560 nm. Superoxide scavenger inhibits the formation of a red dye formazan [90, 91, 92].



Nitroblue tetrazolium

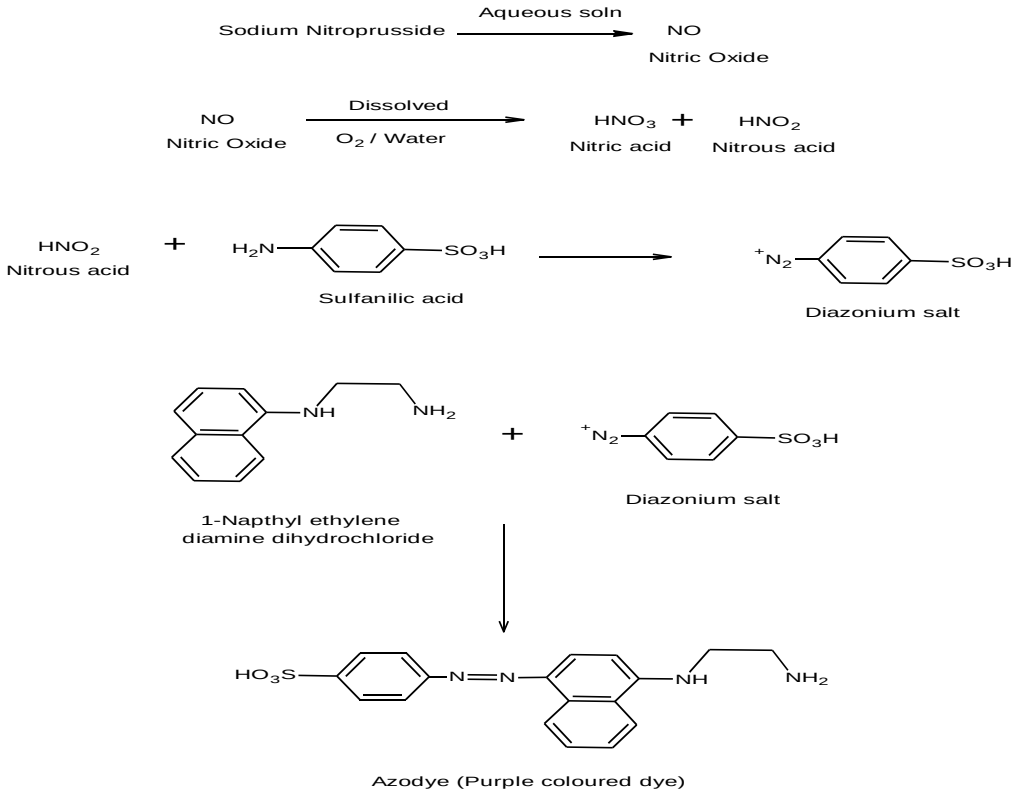
Formazan (Red dye)

Preparation of extract and standard solutions: Accurately 14 mg each of the extracts were weighed and dissolved separately in 3 ml of freshly distilled DMSO. These solutions were serially diluted with DMSO to obtain lower dilutions.

Procedure: To the reaction mixture containing 0.1 ml of NBT (1 mg/ml solution in DMSO) and 0.3 ml of the extracts, the compound and standard in DMSO, 1 ml of alkaline DMSO (1 ml DMSO containing 5 mM NaOH in 0.1 ml water) was added to give a final volume of 1.4 ml and the absorbance was measured at 560 nm.

4.9.3 Nitric oxide radical scavenging assay

Sodium nitroprusside in aqueous solution at physiological pH, spontaneously generates nitric oxide, which interacts with oxygen to produce nitrite ions, which can be estimated by the use of modified Griess Ilosvay reaction [93, 94]. In the present investigation, Griess Ilosvay reagent was modified by using naphthyl ethylene diamine dihydrochloride (NEDD) (0.1% w/v) instead of 1-naphthylamine (5%). Nitrite ions react with Griess reagent, which forms a purple azo dye. In presence of test compounds, likely to be scavengers, the amount of nitrite ions will decrease. The degree of decrease in the formation of purple azo dye will reflect the extent of scavenging. The absorbance of the chromophore formed was measured at 540 nm.



Reagents

1. Sodium nitroprusside solution (10 mM): Accurately 0.30 g of sodium nitroprusside was weighed and dissolved in distilled water and the volume was made up to 100 ml in a volumetric flask.

2. Naphthyl ethylene diamine dihydrochloride (0.1%): Accurately 0.1 g of NEDD was weighed and dissolved in 60 ml of 50% glacial acetic acid by heating and the volume was made up to 100 ml with distilled water in a volumetric flask.

3. Sulphanilic acid reagent (0.33% w/v): Accurately 0.33 g of sulphanilic acid was weighed and dissolved in 20% glacial acetic acid by heating and the volume was made up to 100 ml in a volumetric flask.

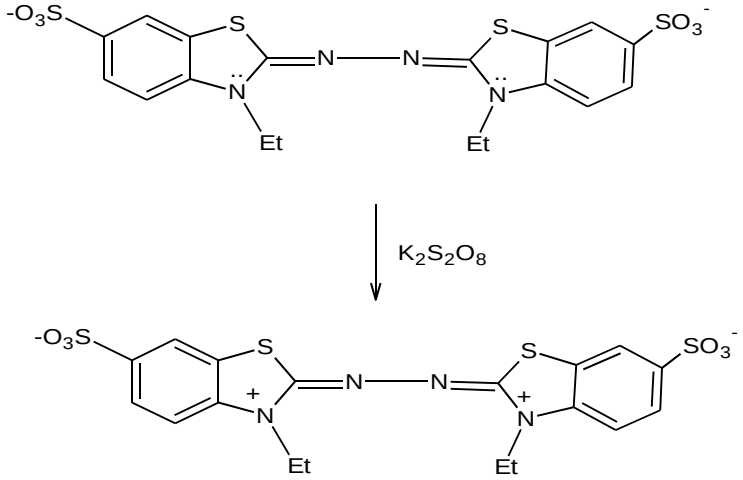
Preparation of extract and standard solutions: These solutions were prepared as described in the DPPH scavenging assay.

Procedure: The reaction mixture (6 ml) containing sodium nitroprusside (10 mM, 4 ml), phosphate buffer saline (PBS, pH 7.4, 1 ml) and extract or standard (1 ml) in DMSO at various concentrations was incubated at 25°C for 150 min. After incubation, 0.5 ml of the reaction mixture containing nitrite ion was removed, 1 ml of sulphanilic acid reagent was added to this, mixed well and allowed to stand for 5 min for completion of diazotization. Then, 1 ml of NEDD was added, mixed and allowed to stand for 30 min in diffused light. A pink coloured chromophore was formed. The absorbance was measured at 540 nm.

4.9.4 ABTS radical scavenging activity

ABTS assay involves a more drastic radical, chemically produced and is often used for screening complex antioxidant mixtures such as plant extracts, beverages and biological fluids. The solubility in both the organic and aqueous media and the stability in a wide pH range raised the interest on the use of ABTS radical cation ($ABTS^{\cdot+}$) for the estimation of the antioxidant activity [95].

Preparation of extract and standard solutions: Accurately 13.5 mg of each of the extracts and the standards, ascorbic acid and rutin were weighed separately and dissolved in 2 ml of freshly distilled DMSO. These solutions were serially diluted with DMSO to obtain lower dilutions.



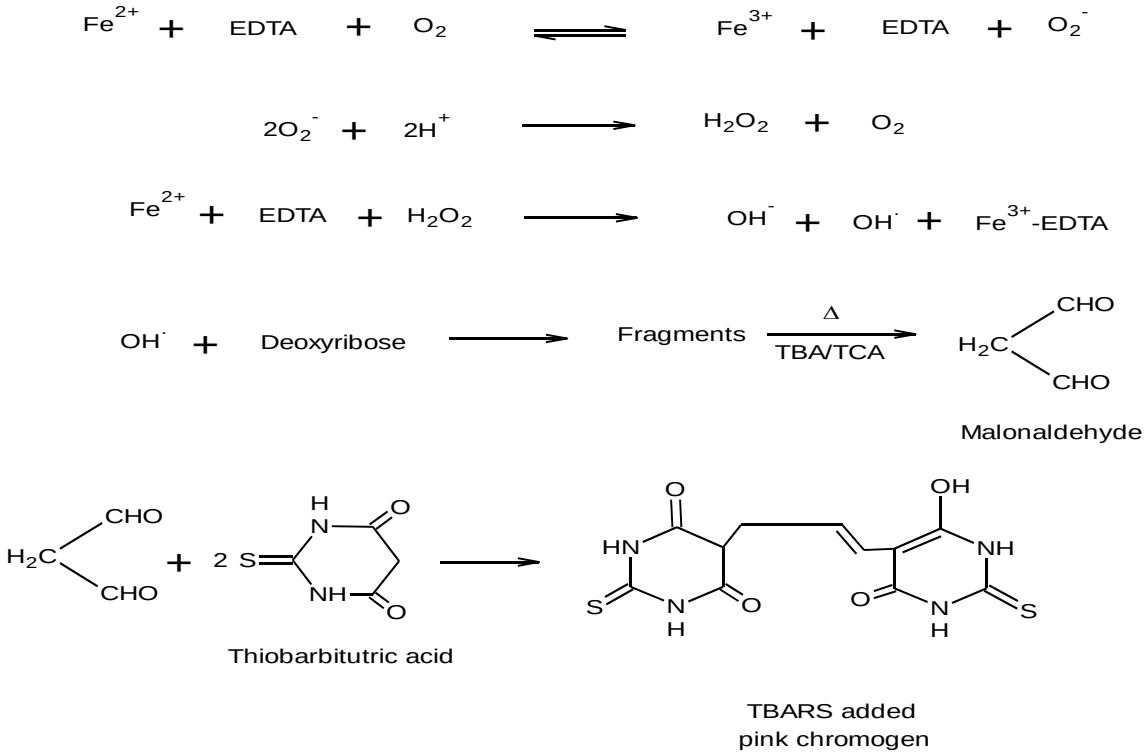
ABTS radical cation

Procedure: Accurately 54.8 mg of ABTS was weighed and dissolved in 50 ml of distilled water (2 mM). Potassium persulphate (17 mM, 0.3 ml) was then added. The reaction mixture was left to stand at room temperature overnight in dark before usage. To 0.2 ml of various concentrations of the extracts or standards, 1.0 ml of distilled DMSO and 0.16 ml of ABTS solution were added to make the final volume to 1.36 ml. Absorbance was measured after 20 min at 734 nm.

4.9.5 Hydroxyl radical scavenging assay by deoxy ribose degradation method

The sugar deoxyribose was degraded on exposure to hydroxyl radical generated by irradiation or by Fenton systems. If the resulting complex mixture of products is heated under acid conditions, malonaldehyde was formed and may be detected by its ability to react with thiobarbituric acid (TBA) to form a pink chromogen [96, 97].

Preparation of extract and standard solutions: Accurately 16 mg of each of the extracts and standard BHA were weighed and separately dissolved in 2 ml of freshly distilled DMSO. These solutions were serially diluted with DMSO to obtain lower dilutions.



Procedure: Various concentrations of the extracts, the compound and standard in DMSO (0.2 ml) were added to the reaction mixture containing deoxyribose (3 mM, 0.2 ml), ferric chloride (0.1 mM, 0.2 ml), EDTA (0.1 mM, 0.2 ml), ascorbic acid (0.1 mM, 0.2 ml) and hydrogen peroxide (2 mM, 0.2 ml) in phosphate buffer (PH 7.4, 20 mM) to give a total volume of 1.2 ml. The solutions were then incubated for 30 min at 37°C. After incubation, ice cold trichloroacetic acid (0.2 ml, 15% w/v) and thiobarbituric acid (0.2 ml, 1% w/v) in 0.25 N HCl were added. The reaction mixture was kept in a boiling water bath for 30 min, cooled and the absorbance was measured at 532 nm.

4.10 *In Vitro α-glucosidase* inhibition activity

Isolation of *α-glucosidase* enzyme from rat small intestine

A male rat (200 g) was sacrificed by cervical dislocation. The small intestine was obtained and flushed several times with ice-cold NaCl (0.9% w/w). The intestine was cleaned from adipose tissue and cut longitudinally. The mucosa was scraped with a glass slide on an ice-cold glass surface. The obtained material containing α glucosidase was homogenized with 20 ml of sodium phosphate buffer and stored at −25^{0}C until used. Total protein content was determined by the Lowry method [98].

Determination of *α-glucosidase* inhibition

To all the test tubes 0.5 ml of Sodium phosphate buffer (80 Mm), pH 7.0 containing 37 mM sucrose was taken and to the test tubes 1 ml of various concentrations of test sample and standard was added. For the control and blank wells 1 ml of phosphate buffer pH 7.0 was added. The reaction was initiated by adding 50µl of crude enzyme to all the tubes except blank.

All the samples were incubated at 37°C for 20 min. The reaction was then stopped by heating the test tubes at 95°C for 1.5 min. The liberated glucose was measured using commercial glucose kit. The percentage inhibition was calculated by using the following formula.

% inhibition = 100 - [A_{sample} / $A_{control}$ x 100]

A_{sample} = absorbance of the sample,

$A_{control}$ = absorbance of the control

4.11 *In vivo* Antidiabetic activity

All the glucose lowering agents available today for treatment of diabetes resulted from *in vivo* anti-diabetic drug discovery approach. The ethyl acetate extract has significant activity in *in vitro* antidiabetic experiment [99, 100]. Hence ethyl acetate extract was selected for *in vivo* antidiabetic experiment.

4.11.1 Animals and treatment

Wistar rats of either sex weighing 180-220g (6 to 8 weeks) with no prior drug treatment were used for the present experiment. The animals were fed with standard laboratory chow (Amrut laboratory Animal feeds, Pranav Agro industries Ltd, Sangli) and provided water *ad libitum* [101]. Animal experiment was performed in the department of pharmacology, J.S.S college of pharmacy, Ootacamund, after approval from the Institutional Animal Ethics Committee (registration number 118/1999/CPCSEA) and animal care was taken as per the guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA)(PH. D/PH. CHEM/03/2009-2010).

4.11.2 Acute toxicity studies

The acute toxicity study of the ethyl acetate extract was determined according to the OECD guidelines No.425. Female wistar rats weighing 180 – 220g (6 to 8 weeks) were used for this study. The general procedure was as follows: one rat was dosed at 400 mg/kg body weight and if no mortality or over toxicity occurred within 48 h, another rat was dosed at 800 mg/kg body weight. In the absence of toxicity, a third rat was dosed at 2000 mg/kg body weight and if again no evidence of toxicity was observed, two additional rats were dosed at this level. In all cases the dosing volume was fixed at 10 ml/kg body weight. The rats were observed for clinical signs of toxicity at 0-0.5, 0.5-1, 1-2, 2-4 and 4-8 h post dosing. The body weights of all the rats were recorded prior to the administration of test sample and at 7 and 14 days post dosing The animals were observed for 24 hours and monitored for 14 days to record general behaviour and mortality [102]. No mortality was observed till the end of the study.

4.11.3 Induction of diabetes

Streptozotocin was dissolved in sterilized citrate buffer pH 4.5. The wistar rats were fast overnight and Streptozotocin 55mg/kg b.w was administered intraperitoneally. After a period of 7 days blood glucose was estimated to confirm the diabetes. The rats were maintained for a period of 14 days to stabilize the diabetic condition. The rats with blood glucose level above 200 mg/dl were considered diabetic and used in the experiment [103, 104].

4.11.4 Experimental protocol

The animals were divided into following groups. Each group contain 5 animals [105].

Group 1 - Untreated Control

Group 2 - Diabetic Control

Group 3 - Positive Control (glibenclamide 10 mg/kg body weight)

Group 4 - Diabetic rats given (100 mg/kg b.w) ethyl acetate extract

Group 5 - Diabetic rats given (200 mg/kg b.w) ethyl acetate extract

Group 6 - Diabetic rats given (400 mg/kg b.w) ethyl acetate extract

The ethyl acetate extract was administered orally, twice daily for 7days and biochemical parameters were estimated [106].

4.11.5 Determination of serum biochemical parameters

Blood was collected by retroorbital sinus. Blood samples were centrifuged at 4300 rpm for 20 min to obtain serum. Serum biochemical parameters were estimated using biochemical kits according to instructions.

5. RESULTS AND ANALYSIS

5.1 Plant material and extraction

The plant material of *Actiniopteris radiata* was extracted successively with petroleum ether, chloroform, ethyl acetate and ethanol. The yield of these extracts are 2.0, 1.2, 2.2 and 3.2 % w/w respectively.

5.2 Preparation of plant extract

The extractive values of successive extracts of *Actiniopteris radiata* is given in Table 2.

Table 2. Extractive values of *Actiniopteris radiata*

S.No.	Solvent extracts	% w/w of extracts
1	Pet. ether	2.0
2	Chloroform	1.2
3	Ethyl acetate	2.2
4	Ethanol	3.2

5.3 Preliminary phytochemical studies

Preliminary phytochemical studies revealed that presence of steroids, glycosides, carbohydrates, flavonoids and fatty acids. The results are given in the Table 3.

Table 3. Phytochemical analysis of extracts of *Actiniopteris radiata*

Phytoconstituents	Pet.ether	Chloroform	Ethyl acetate	Ethanol
Alkaloids	-	-	-	-
Saponins	-	-	-	-
Carbohydrates	-	+	-	-
Glycosides	-	-	+	+
Steroids	+	+	-	-
Fatty acids	+	-	-	-
Flavanoids	-	-	+	+

Physicochemical analysis

5.4.1 Ash value

The percentage of total, water soluble and acid insoluble ash values of the plant was found to be 14.88 ± 1.4, 5.24 ± 0.5, 0.51 ± 0.05 % w/w, respectively.

5.4.2 Extractive value

The percentage of water soluble and alcohol soluble extractive values of the plant powder was found to be 6.27 ± 0.2% w/w and 2.29 ± 0.3% w/w, respectively.

5.4.3 Moisture content

The moisture content of the plant powder was found to be 2.30 ± 0.2 % w/w.

Table 4: Physicochemical analysis of Actiniopteris radiata

Parameter	**Evaluation**	**Value (%w/w)**
Ash value	Total ash	14.88 ± 1.4
	Water insoluble ash	5.24 ± 0.5
	Acid insoluble ash	0.51 ± 0.05
Extractive value	Water soluble extractives	6.27 ± 0.2
	Alcohol soluble extractives	2.29 ± 0.3
Moisture content		2.30 ± 0.2

Values are mean ± SD, n=3

5.5 Quantitative phytochemical analysis

The quantitative analysis of petroleum ether, chloroform, ethyl acetate and ethanol extracts for flavonoids and phenolic compounds are given in Table 5. Among the extracts, the ethyl acetate extract shows the highest concentration of flavonoids and phenolic compounds (0.059 ± 0.05 and 0.10 ± 0.08µg/ml, respectively).

Table 5: Quantitative estimation of flavonoids and phenolic compounds in plant extracts of *Actiniopteris radiata*

Sample	**Concentration (µg/ml)**	
	Phenolic compounds	**flavonoids**

Pet. ether extract	-	-
Chloroform extract	0.084 ± 0.08	0.029 ± 0.02
Ethyl acetate extract	0.104 ± 0.08	0.059 ± 0.05
Ethanol (50 %) extract	0.098 ± 0.01	0.030 ± 0.02

Values are mean ± SD, n=3.

5.6 Isolation of compounds and characterization

The column chromatography of ethyl acetate extract of *Actiniopteris radiata* yielded 2 new compounds. Compound 1 is 2-(3, 4-O–Diglucos cinnamoyl) – 4-hydroxyl furan and compound 2 is 1-heptaloyl, 8-hexyl, 3-(O-diglucos), 10-methyl, 9, 10–dihydro naphthalene. These two compounds were characterized by TLC, melting point, UV, IR, NMR and Mass spectroscopy.

5.6.1 Compound 1

In the ^{1}H-NNR spectrum the signals at δ 6.70 (H-2) and 7.30 (H-3) are protons of –C=C-. They have a cross peak in the ^{1}H-^{1}H COSY spectrum. The signals at δ 5.70 (H-6) and 7.56 (H-8) are meta to each other and belongs to the furan ring. The signal at δ 7.11 (H-7) indicates the proton of hydroxyl group. The signal at δ 5.70 is in the upfield because it is between two carbon atoms C-5 and C-7 which contains hydroxyl group. Similarly the proton at H-8 appeared at δ 7.56. The signals at δ 6.80 (H-5^{1}), 7.10 (H-6^{1}) and at δ 6.25 (H-2^{1}) indicates protons of aromatic ring. They have cross peaks with each other in the ^{1}H-^{1}H COSY spectrum.

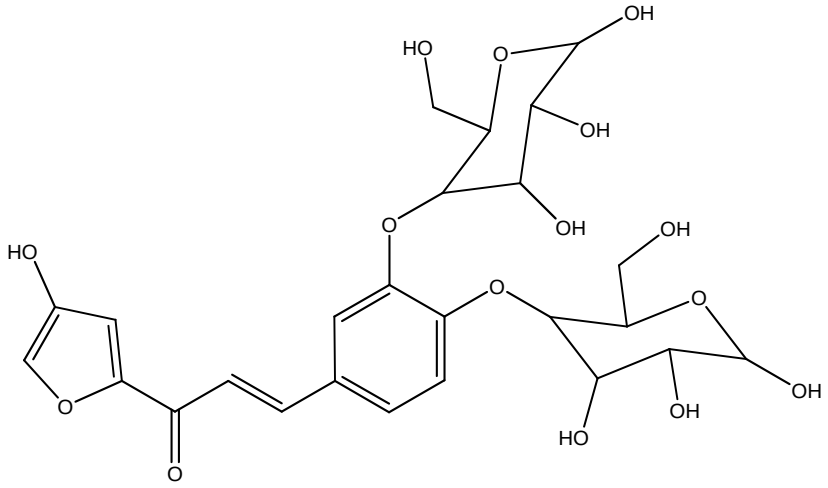
The ^{13}C-NMR spectrum has a signal at δ 171.48 for a carbonyl carbon, five signals at δ 166.60 (C-5), 150.28 (C-4^{1}), 116.92 (C-7), 147.23 (C-3^{1}) and 128.93 (C-1^{1}) are quaternary in nature. It indicates that two signals at 101.47 and 104.21 due to two anomeric carbon atoms C-1^{11} and C-1^{111} respectively. It was supported by the appearance of two anomeric hydrogen signals in ^{1}H-NMR at δ 5.30 (H-1^{11}) and 4.56 (H-1^{111}). The signals between δ 3.40 to 4.00 in the ^{1}H-NMR and between δ 62.00 to 78.64 in ^{13}C-NMR suggest the presence of two glycoside moieties in the compound. The glycosides are attached to a furochalcone nucleus. The above data suggests that it is a chalcone with two glycosidic moieties (two hexoses).

Table 6. NMR Spectral data of compound 1

Carbon	Signal (δ)	DEPT 135	Proton	Signal (δ)
2	117.59	up	H-2	6.70 *d*, 1H, *J*=16 Hz
3	136.86	up	H-3	7.30 *d*, 1H, *J*=16 Hz
4	171.48	--		
5	166.60	--		
6	92.55	up	H-6	5.70 *d*, 1H, *J*=2Hz
7	116.92	--	H-7	7.11 *d*, 1H, *J*=2Hz
8	161.56	up	H-8	7.56 *d*, 1H, *J*=2Hz
Aromatic carbon and Hydrogen				
1^{1}	128.93			
2^{1}	100.86	up	$H\text{-}2^{1}$	6.25 *d*, 1H, *J*=2Hz
3^{1}	147.23	-		
4^{1}	150.28			
5^{1}	117.40	up	$H\text{-}5^{1}$	6.80 *d*, 1H, *J*=7Hz
6^{1}	125.67	up	$H\text{-}6^{1}$	7.10 *d*, 1H, *J*=7Hz
Glycosidic carbon and Hydrogen				
Carbon	Signal (δ)	Proton	Signal	
1^{11}	101.47	$H\text{-}1^{11}$	5.30	
2^{11}	78.64	$H\text{-}2^{11}$	3.50	
3^{11}	74.90	$H\text{-}3^{11}$	3.48	
4^{11}	71.64	$H\text{-}4^{11}$	3.50	
5^{11}	77.62	$H\text{-}5^{11}$	3.52	
6^{11}	62.64	$H\text{-}6^{11}$	3.71, 3.90	
1^{111}	104.21	$H\text{-}1^{111}$	4.56	
2^{111}	78.52	$H\text{-}2^{111}$	3.50	
3^{111}	74.89	$H\text{-}3^{111}$	3.52	
4^{111}	70.95	$H\text{-}4^{111}$	3.40	
5^{111}	77.61	$H\text{-}5^{111}$	3.52	
6^{111}	62.21	$H\text{-}6^{111}$	3.70, 3.98	

The UV spectrum has the maximum absorption at 255, 334 and 374 nm showing the presence of a chalcone system. The IR spectrum has characteristic bands at 3390, 3379, 3369, 3350, (-OH), 1680 (C=O), 1627, 1600 (C=C) and 1080 (C-O) cm^{-1}.

The Mass spectrum has a peak at M/Z 570 for M^+ ion in the negative mode ESI-MS spectrum. The peaks at M/Z 408 for $[M-162]^-$ ion and at M/Z 246 for $[M - 2X162]^-$ ion confirms the presence of two glycosidic moieties. Hence the chemical name of the compound is 2-(3, 4-O–Diglucos cinnamoyl) – 4-hydroxyl furan and structure of the compound is given below.



Molecular formula : $C_{25}H_{30}O_{15}$
Molecular weight: 570.00
Physical properties: Yellow solid, soluble in methanol.
Melting point: 98°C
Thin layer Chromatography:

Solvent system	R_f Values
Methanol:Chloroform – 6:4	0.48
n-Butanol:Glacial.acetic acid:water – 2.5:0.5:2.0	0.52

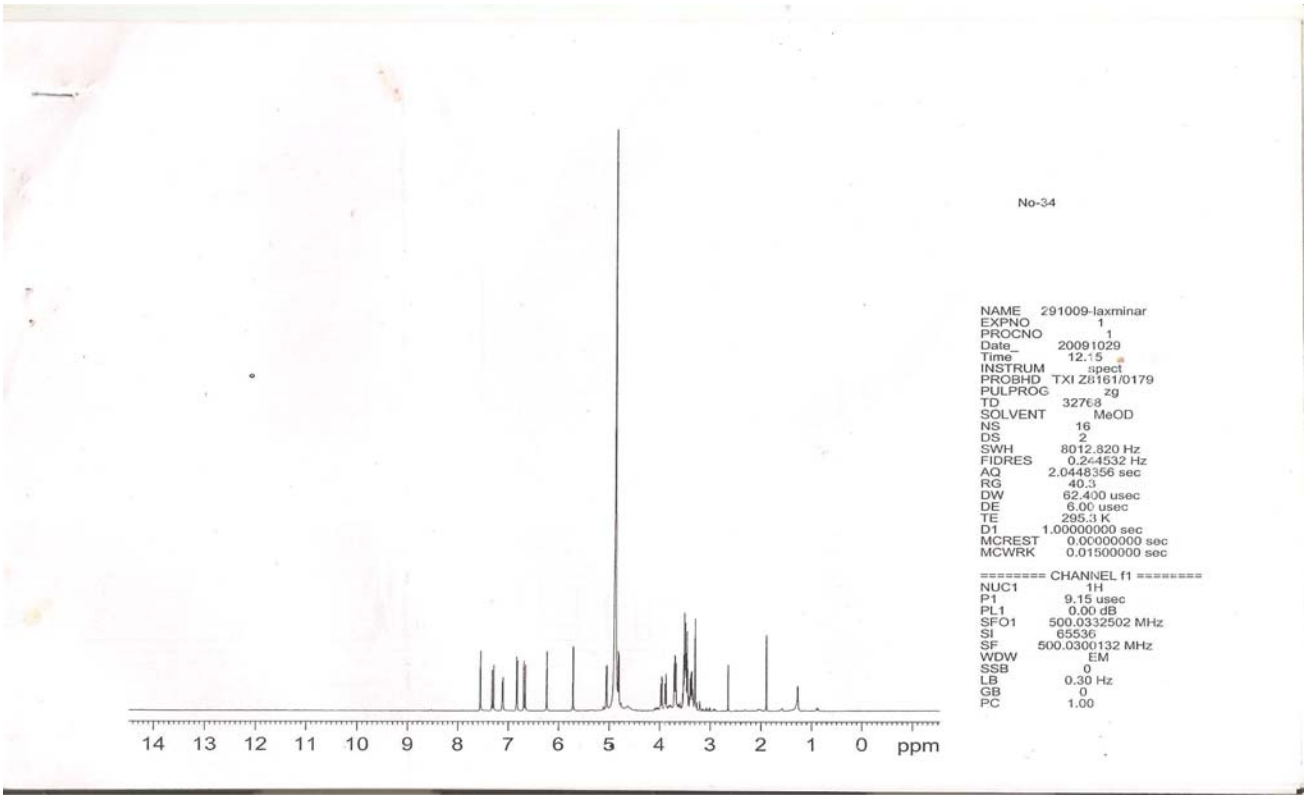


Figure 2. ^{1}H NMR Spectrum of Compound - 1

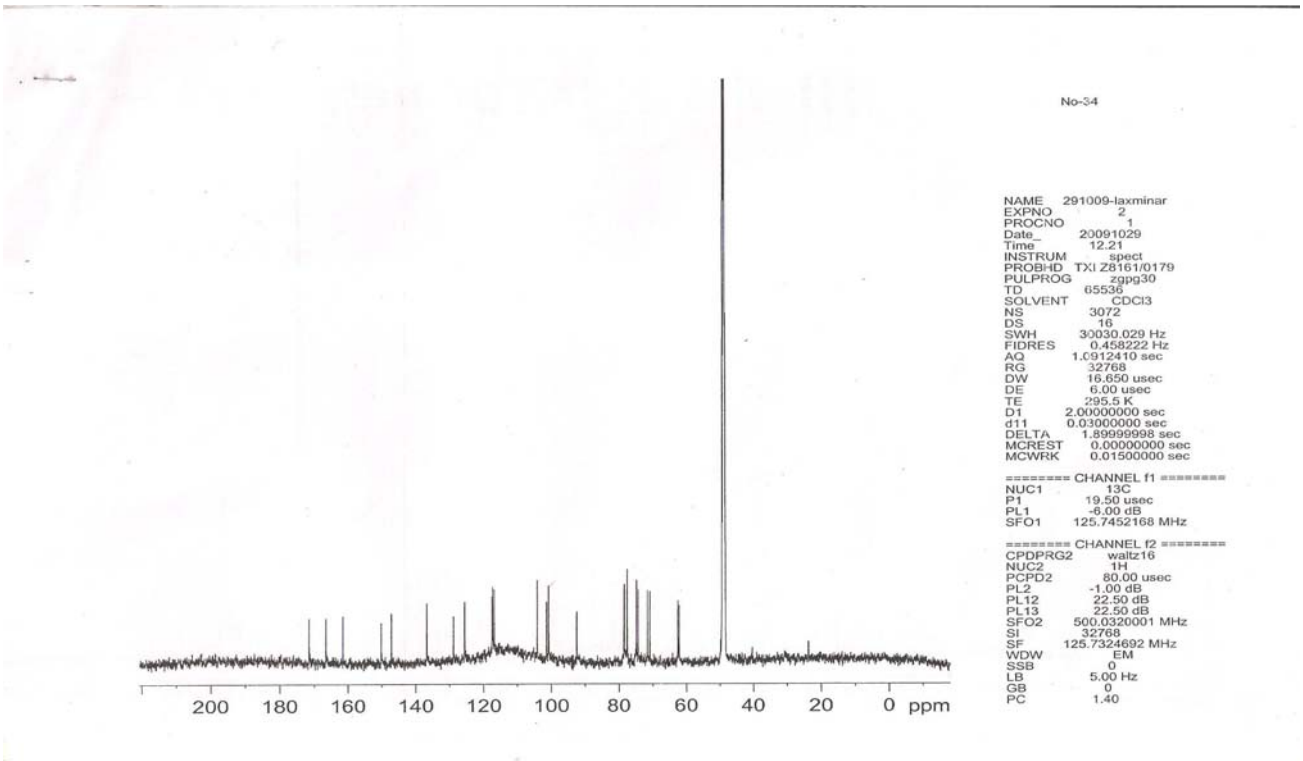


Figure 3. ^{13}C NMR Spectrum of Compound - 1

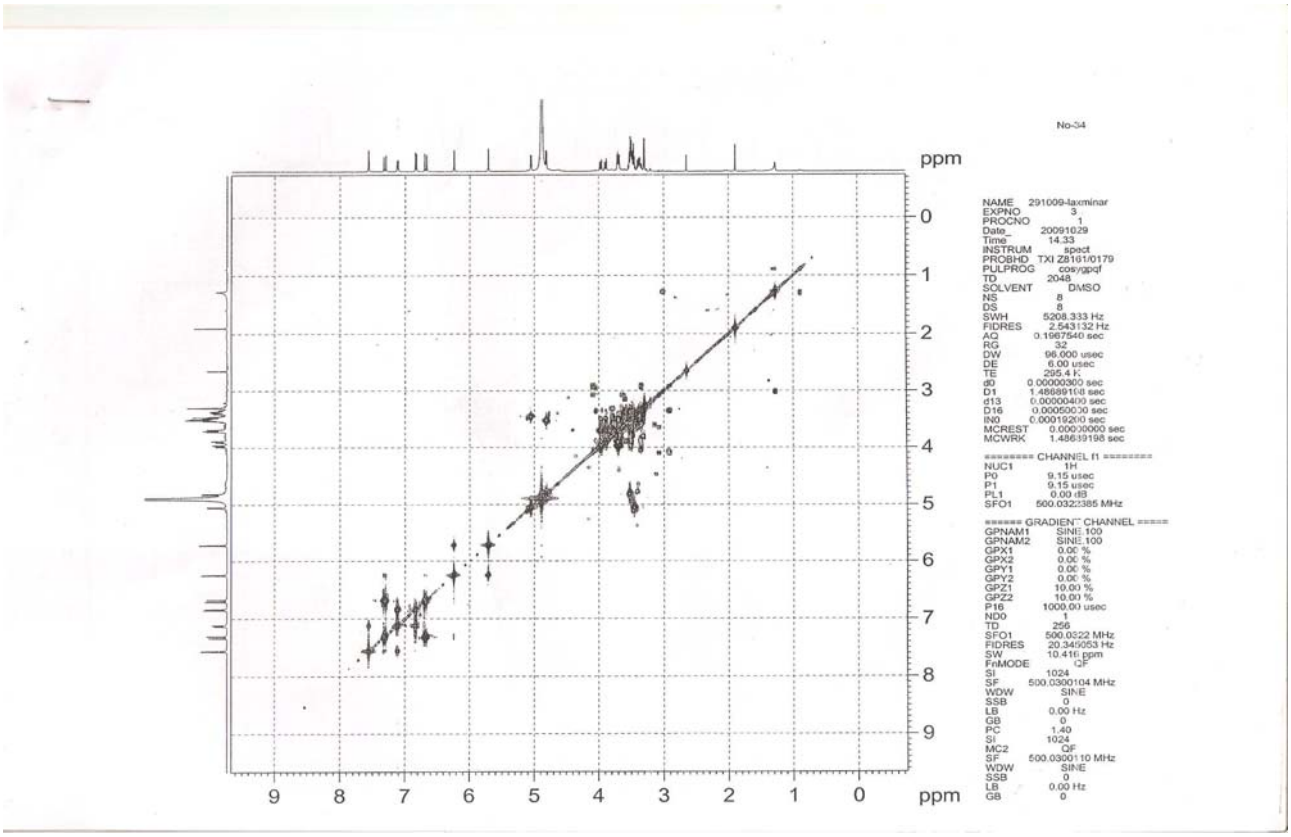


Figure 4. Cosy Spectrum of Compound - 1

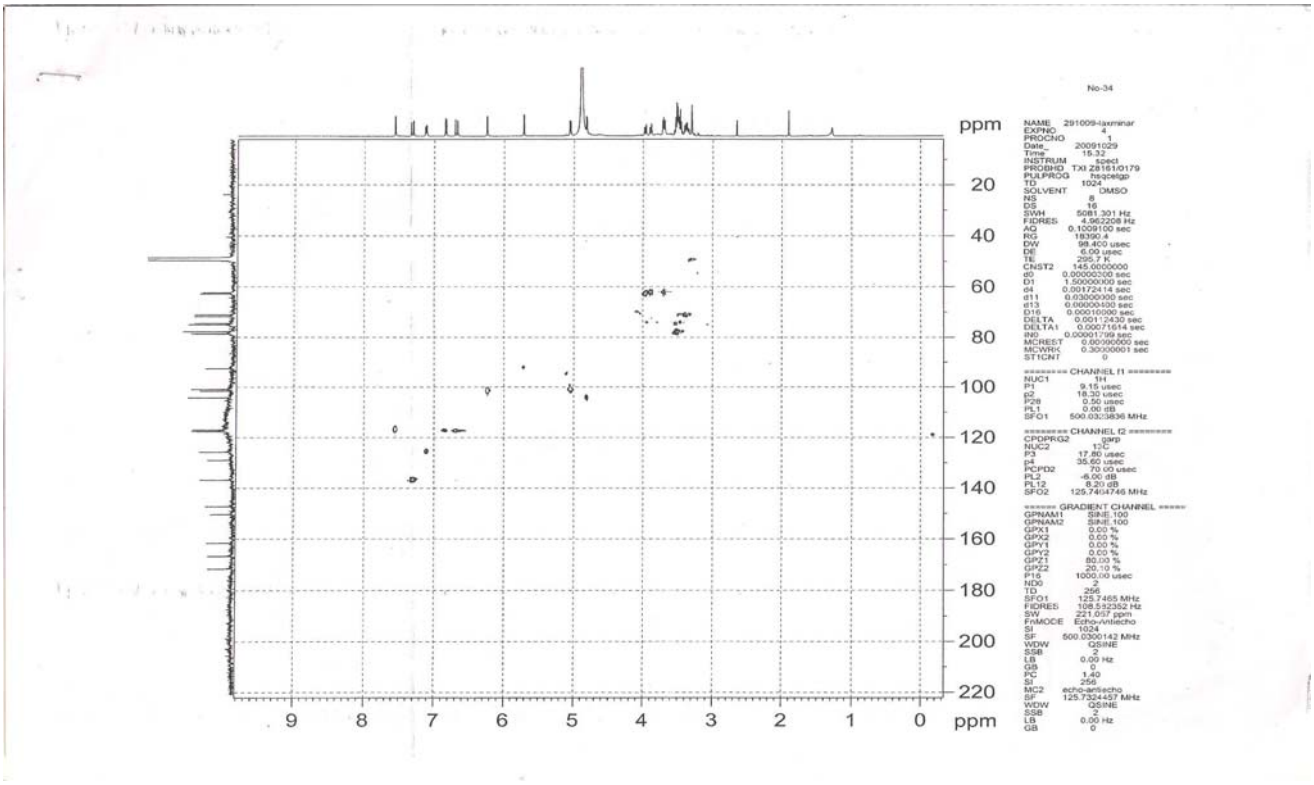


Figure 5. HSQC Spectrum of Compound - 1

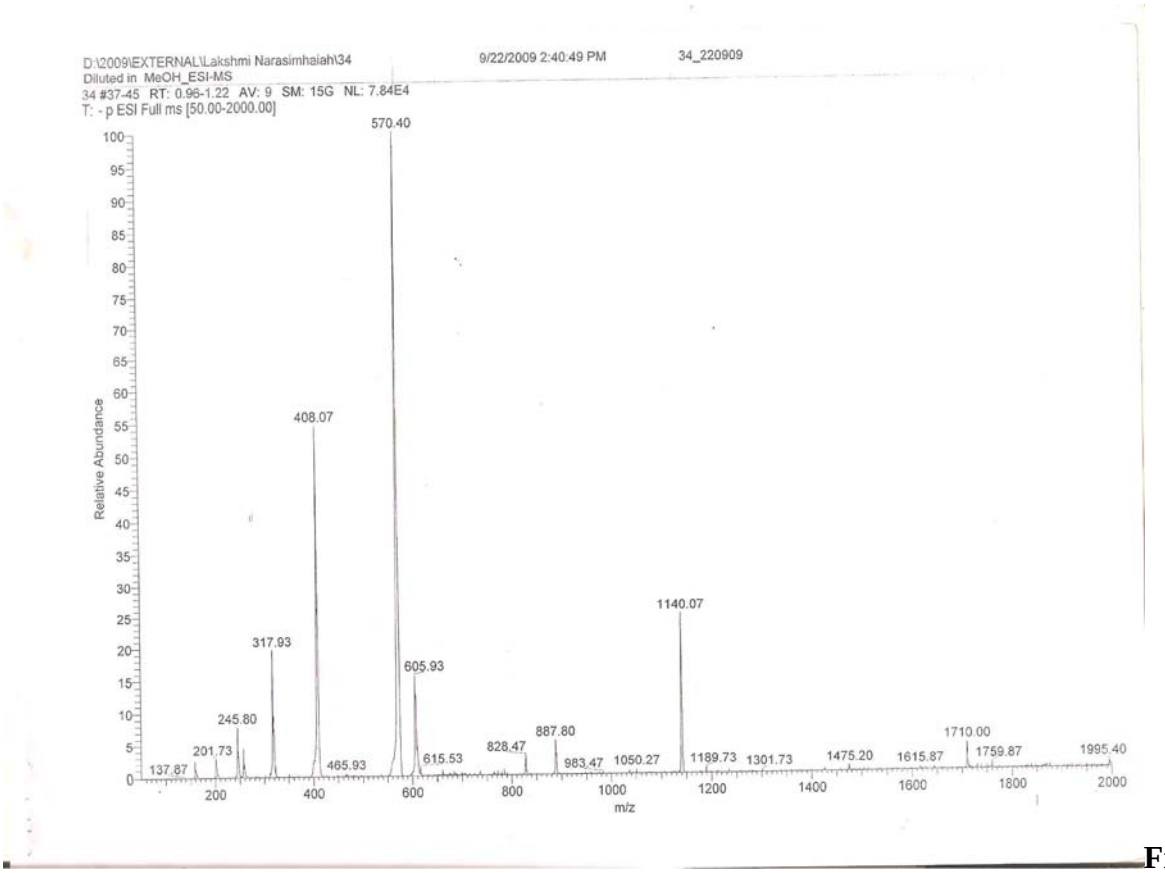


Figure 6. Mass Spectrum of Compound - 1

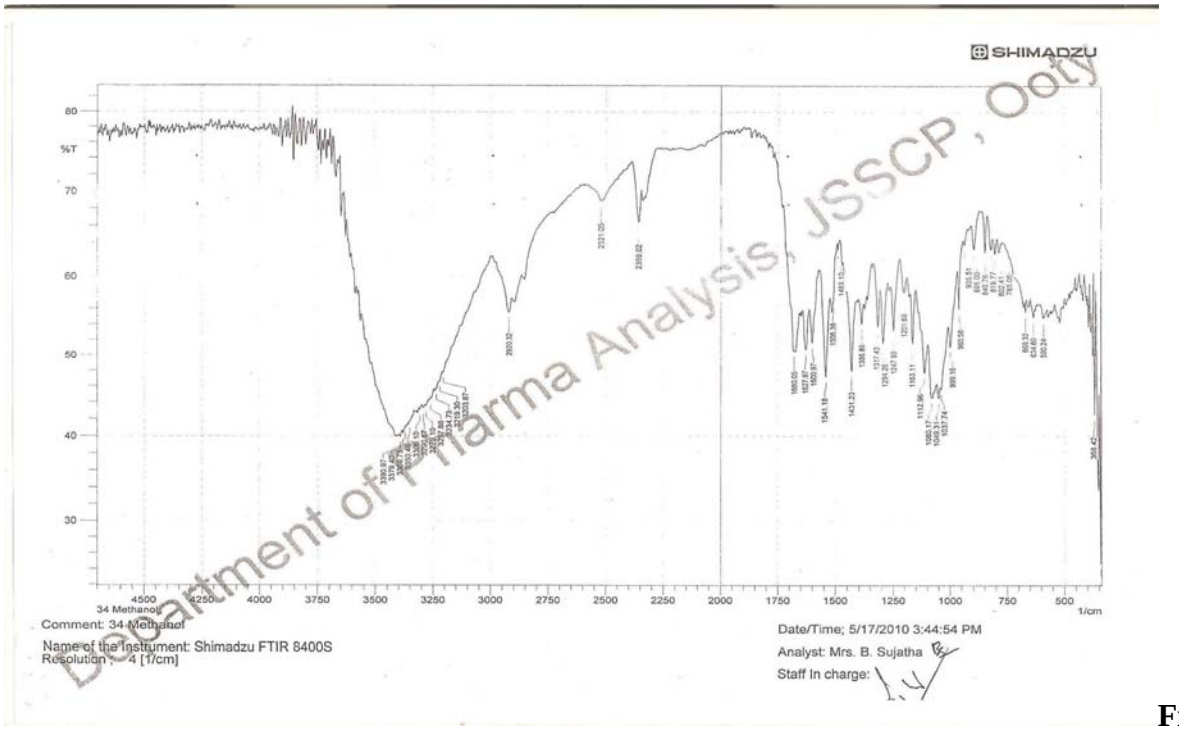
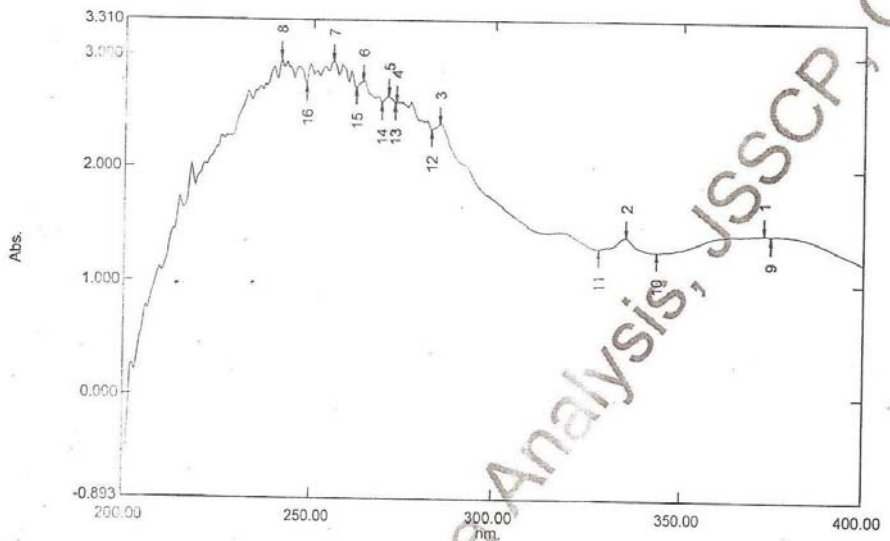


Figure 7. IR Spectrum of Compound - 1

Spectrum Peak Pick Report

05/22/2010 03:21:18 PM

Data Set: 34 uv_113904 - RawData



Measurement Properties	
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Scan Speed:	Fast
Sampling Interval:	0.5
Auto Sampling Interval:	Disabled
Scan Mode:	Auto
Instrument Properties	
Instrument Type:	UV-1700 Series
Measuring Mode:	Absorbance
Slit Width:	1.0 nm
Light Source Change Wavelength:	340.8 nm
S/R Exchange:	Normal
Attachment Properties	
Attachment:	None
Sample Preparation Properties	
Weight:	
Volume:	
Dilution:	
Path Length:	
Additional Information:	

No.	P/V	Wavelength	Abs.	Description
1	P	373.00	1.443	
2	P	334.50	1.413	
3	P	285.50	2.395	
4	P	273.50	2.581	
5	P	271.00	2.635	
6	P	264.00	2.767	
7	P	255.50	2.937	
8	P	241.00	2.960	
9	V	375.00	1.438	
10	V	343.00	1.281	
11	V	327.50	1.308	
12	V	283.00	2.334	
13	V	273.00	2.571	
14	V	269.00	2.581	
15	V	262.00	2.710	
16	V	248.00	2.741	

Figure 8. UV Visible Spectrum of Compound - 1

5.6.2 Compound 2

In ^{1}H-NMR, the multiplet at δ 0.9 for six protons indicates the presence of two methyl groups. They are terminal methyl groups of a long chain hydrocarbon. The two methyl groups suggests the presence of two long chain hydrocarbon groups. This was further supported by a broad singlet at δ 1.29. The complex multiplet signal at δ 2.3 (3H) indicates the presence of a methylene and a methyne group on either side of a carbonyl group. The presence of a two proton multiplet at δ 5.35 and 5.30 for proton adjacent to carbonyl group. Further it has two signals at δ 4.25 and 4.35 each for one proton indicating the presence of two anomeric protons indicates the presence of a disaccharide. This was completed by the signals between δ 3.65 and 4.10. The quateret signal at δ 3.00 for two protons indicates the presence of $-O-CH_2$ group supporting the above data.

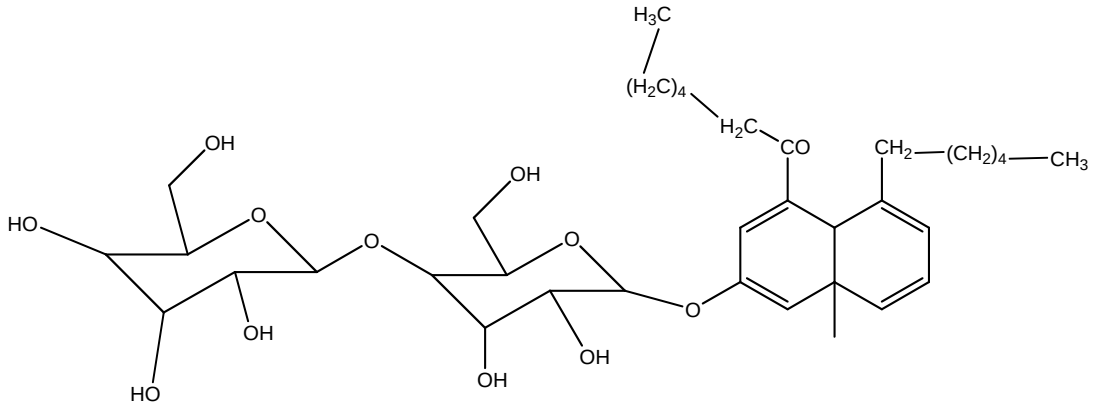
The ^{13}C-NMR spectra has the signals at δ 130.86 and 129.02 for the carbons adjacent to the carbonyl group, two anomeric carbon signals at δ 105.00 and 99.13, a carbonyl carbon at δ 185.00. The presence of signals at δ 11.00, 14.00 and 23.00 indicates the presence of three methyl groups. The group of signals between δ 26.00 and 43.45 indicates the presence of long chain hydrocarbons. The group of signals between δ 77.83 and 62.78 supports the presence of two hexose units. The spectral data of these two hexose units are given below.

C-1^{1}	105.00	C-1^{11}	99.13
C-2^{1}	72.50	C-2^{11}	67.06
C-3^{1}	77.83	C-3^{11}	74.92
C-4^{1}	69.66	C-4^{11}	66.67
C-5^{1}	73.42	C-5^{11}	71.64
C-6^{1}	62.49	C-6^{11}	62.78

The protons and their respective carbon signals were established using HSQC spectra. The position of the groups were fairly established based on the ^{1}H-^{1}H COSY spectra.

1. A cross peak between the signals at δ 5.35 (H-8) and 5.30 (H-7) suggesting that H-8 and H-7 are adjacent to each other.
2. The signal at δ 5.30 (H-7) has a cross peak with a peak at δ 2.75 (H-6). Further there is no cross peaks for the proton H-10 suggesting that C-10 was connected to a quaternary carbon (C-5).

3. The signal at δ 5.35 (H-8) has cross peak with a signal at δ 2.00 (H-9) which in turn has cross peak with a signal at δ 1.60. The cross peaks were observed between signals at δ 1.60, 1.29 and 1.29, 0.90. This strongly suggests that C-8 is connected to a long chain hydrocarbon.
4. The cross peaks were observed between the following signals at δ 3.40 (H-3) and 2.90 (H-2); 2.90 (H-2) and 2.40 (H-1); 2.40 (H-1) and 1.29 (long chain methylene groups); 1.29 and 0.90 (CH_3 – group).
5. The signal at δ 3.40 (H-3) suggests that the methene proton was under oxygen function and two glycoside units are attached to the oxygen at C-3. Hence the chemical name of the compound is 1-heptaloyl, 8-hexyl, 3-(O- diglucos), 10-methyl, 9, 10–dihydro naphthalene and structure of this compound is given below.



The IR spectra has characteristic absorption at 3369, 3329, 3315 (-OH), 1734 (C=O), 1670 (C=C) and at 1035 (C-O) cm^{-1} groups. The UV spectrum has peaks at 256, 318 and 334 nm for unsaturated carbonyl compounds. The positive mode ESI-MS has m/z 680 for M^+ ion and m/z 702 for $[M+Na]^+$ ion.

Physical properties : yellow solid, soluble in methanol.

Molecular formula : $C_{36}H_{56}O_{12}$

Molecular weight : 680.00

Melting point : 136ºC

Thin layer Chromatography:

Solvent system	R_f Values
Methanol:Chloroform – 6:4	0.52
Ethyl acetate:Methanol – 9:1	0.83

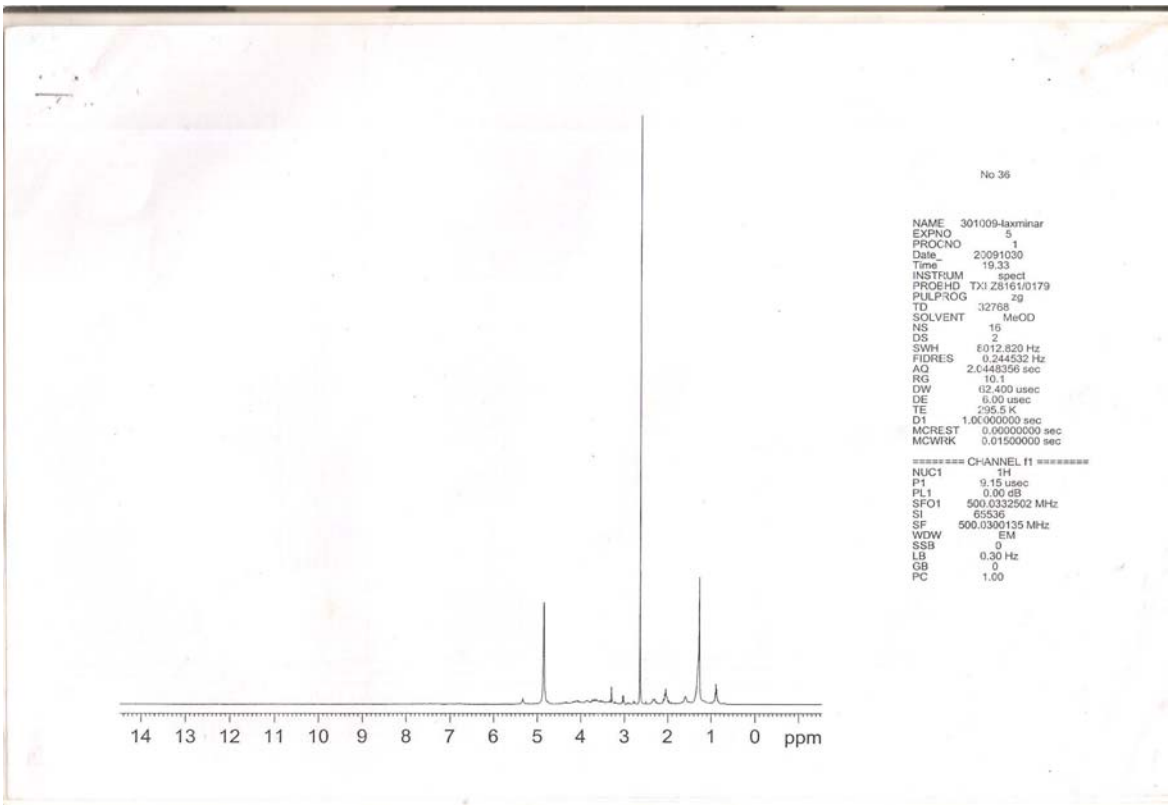


Figure 9. ^{1}H NMR Spectrum of Compound - 2

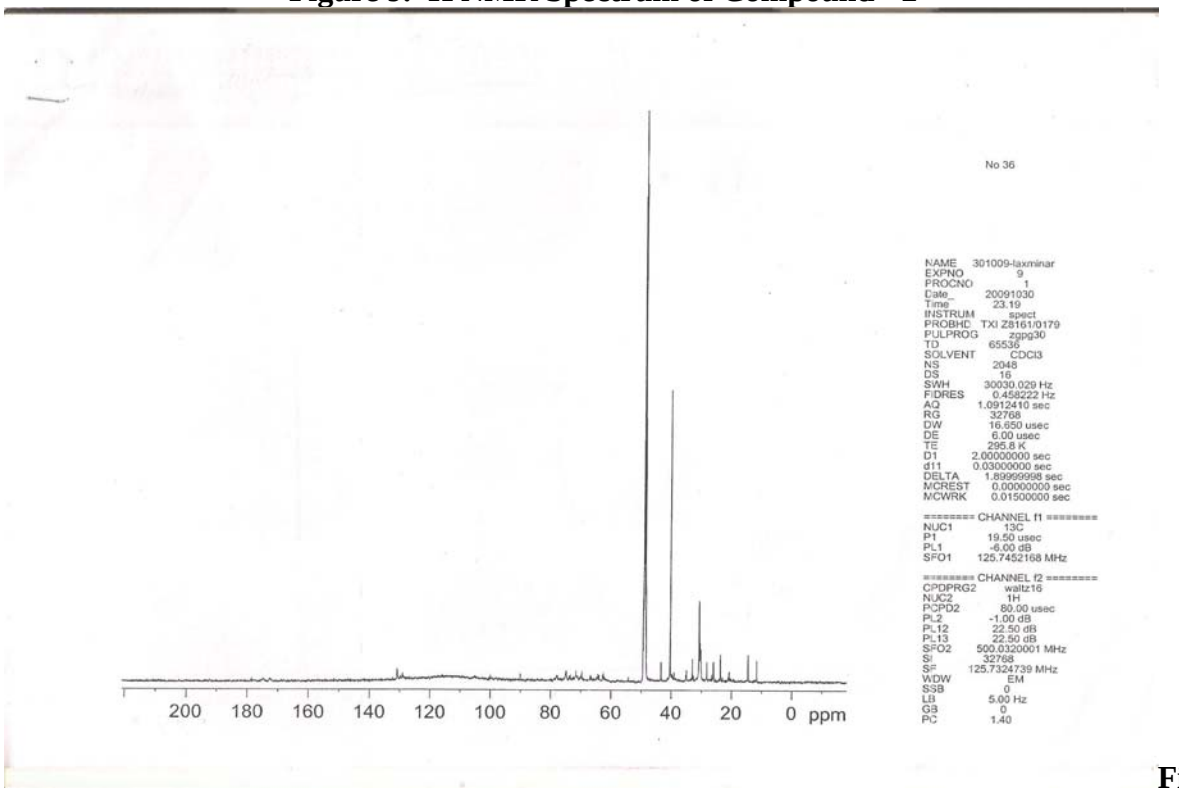


Figure 10. ^{13}C NMR Spectrum of Compound - 2

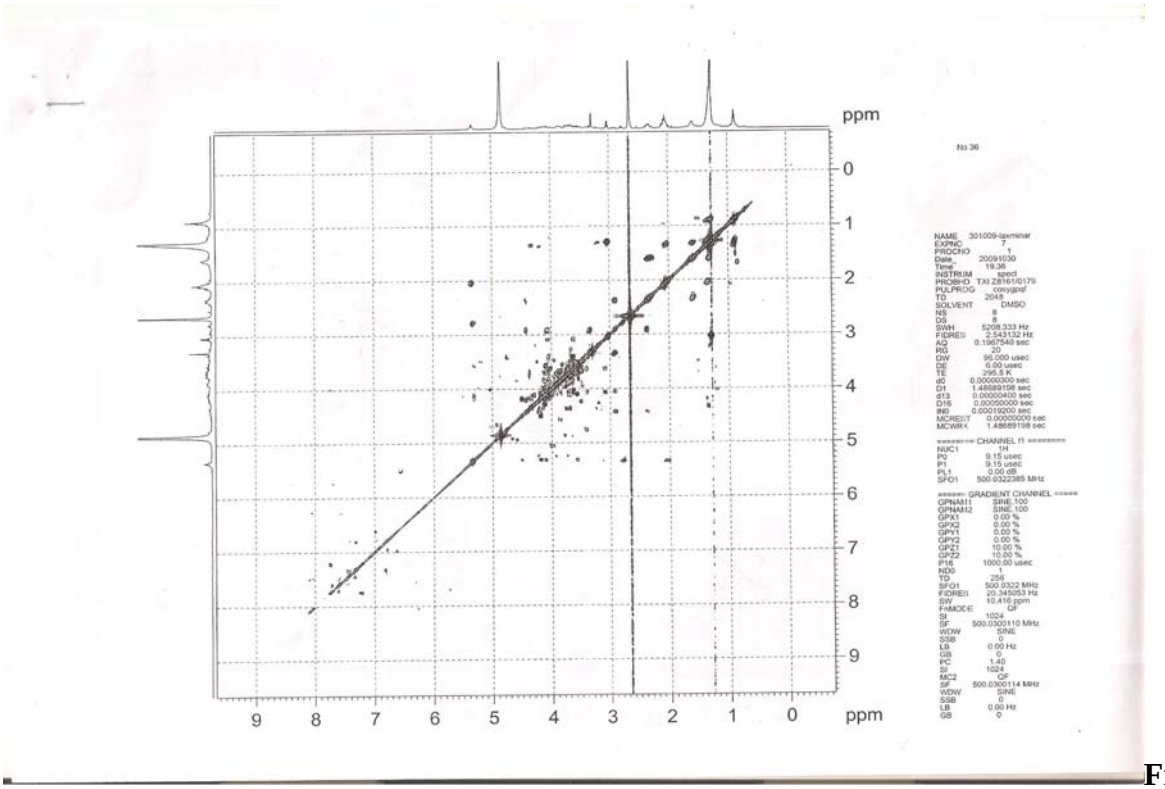


Figure 11. Cosy Spectrum of Compound - 2

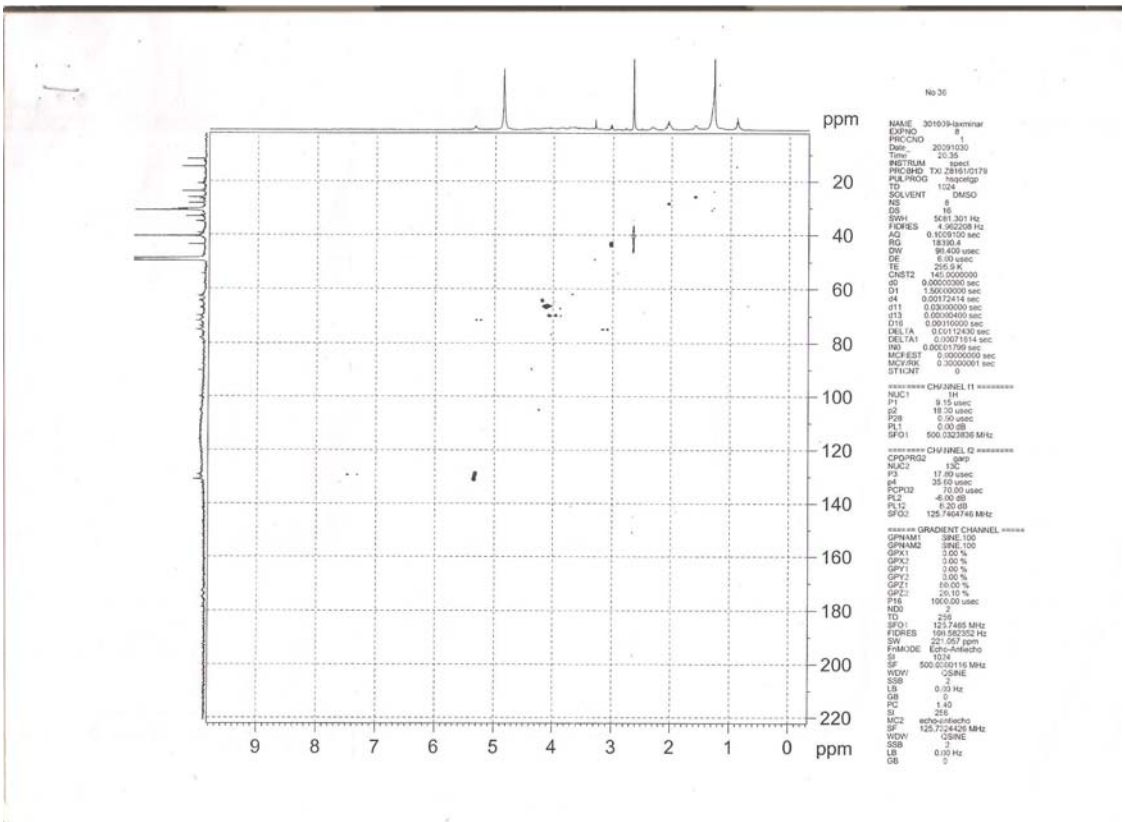


Figure 12. HSQC Spectrum of Compound - 2

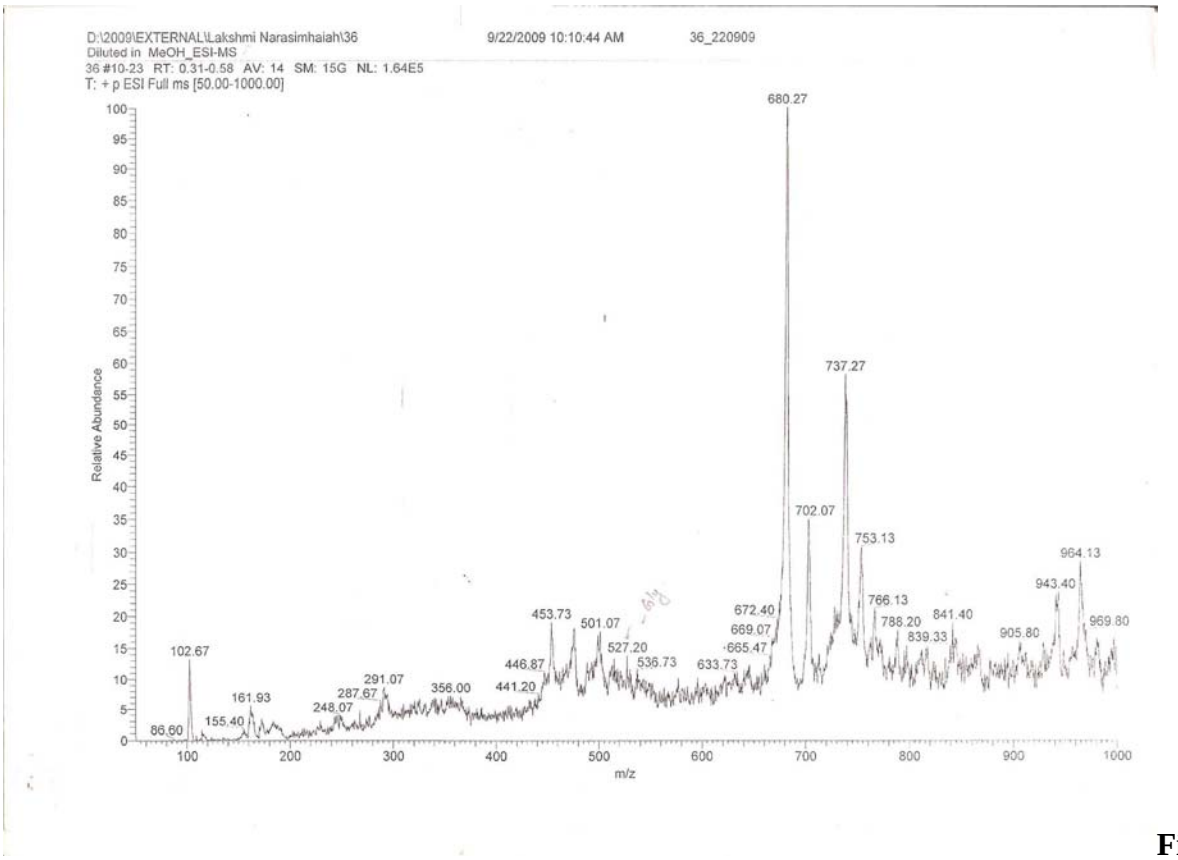


Figure 13. Mass Spectrum of Compound - 2

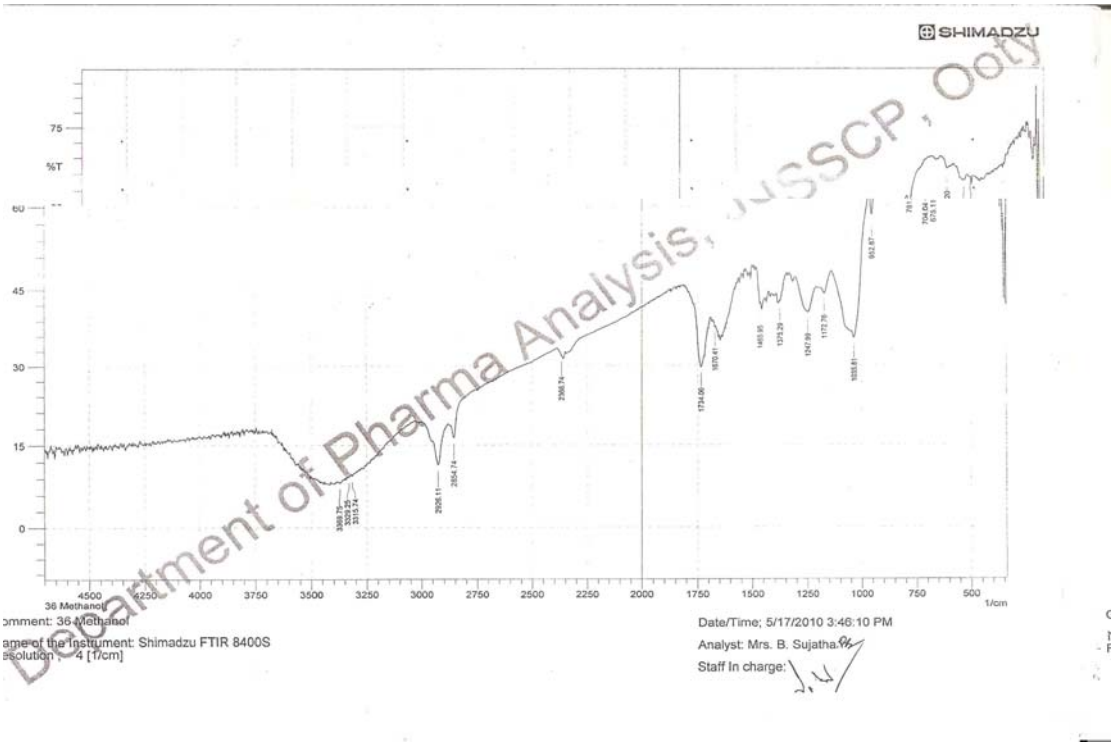
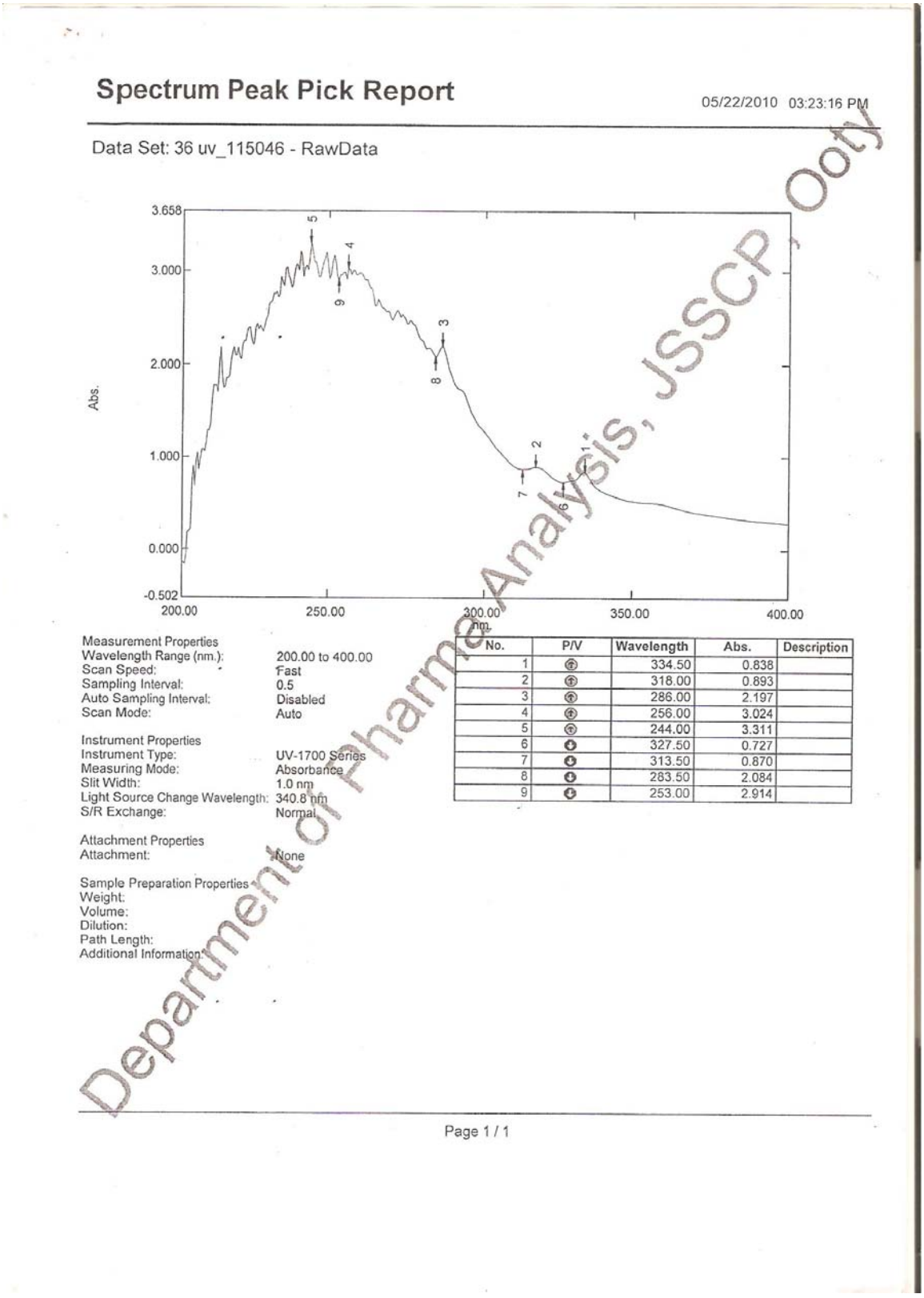


Figure 14. IR Spectrum of Compound - 2



Spectrum Peak Pick Report

05/22/2010 03:23:16 PM

Data Set: 36 uv_115046 - RawData

Measurement Properties	
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Scan Speed:	Fast
Sampling Interval:	0.5
Auto Sampling Interval:	Disabled
Scan Mode:	Auto
Instrument Properties	
Instrument Type:	UV-1700 Series
Measuring Mode:	Absorbance
Slit Width:	1.0 nm
Light Source Change Wavelength:	340.8 nm
S/R Exchange:	Normal
Attachment Properties	
Attachment:	None
Sample Preparation Properties	
Weight:	
Volume:	
Dilution:	
Path Length:	
Additional Information:	

No.	P/V	Wavelength	Abs.	Description
1	↑	334.50	0.838	
2	↑	318.00	0.893	
3	↑	286.00	2.197	
4	↑	256.00	3.024	
5	↑	244.00	3.311	
6	↓	327.50	0.727	
7	↓	313.50	0.870	
8	↓	283.50	2.084	
9	↓	253.00	2.914	

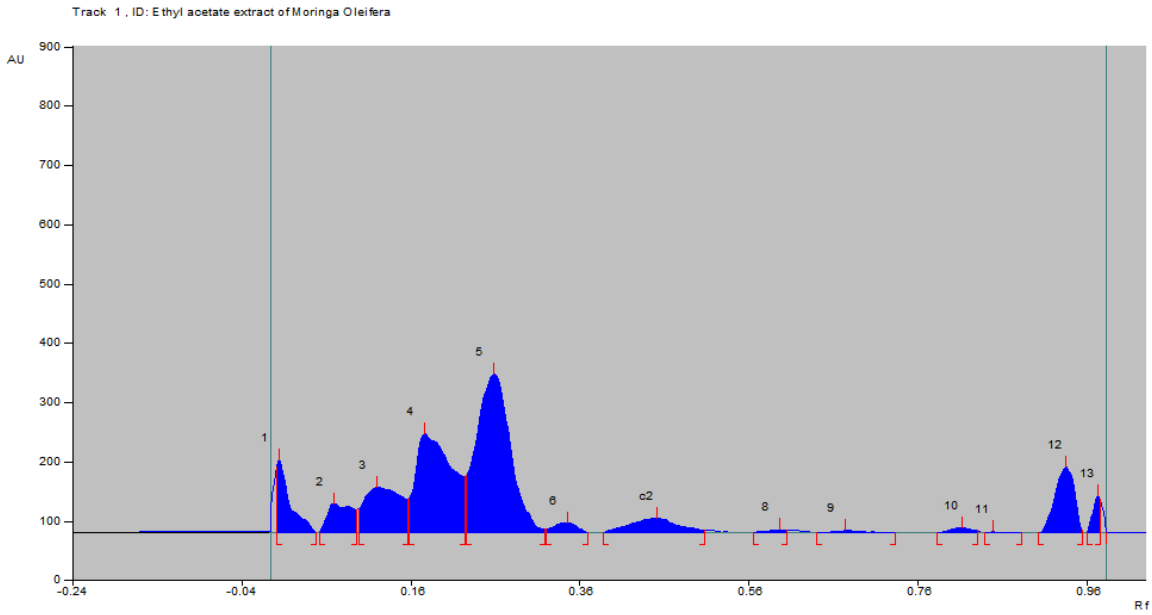
Page 1 / 1

Figure 15. UV Visible Spectrum of Compound - 2

QUALITATIVE AND QUANTITATIVE HPTLC ESTIMATION

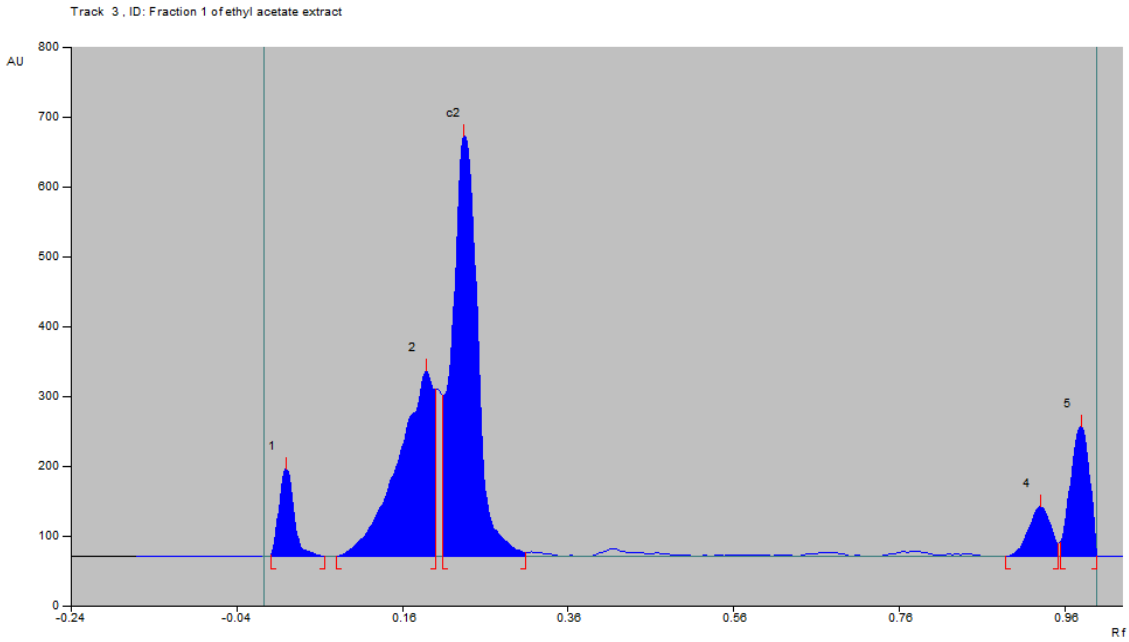
The results of the HPTLC finger printing of ethyl acetate extract and Fraction 1 at 366 nm are given in Figure 16 and 17. The ethyl acetate extract shows 10 well resolved peaks with R_f values of 0.01, 0.07, 0.12, 0.18, 0.26, 0.35, 0.45, 0.60, 0.68, 0.82, 0.85, 0.94 and 0.98. The Fraction 1 shows 5 well resolved peaks with R_f values of 0.02, 0.19, 0.24, 0.93 and 0.98. Among these peaks, the peak corresponding to R_f value of 0.24 has the highest peak area and spectra of this peak matches with the peak with Rf value of 0.26 in the ethyl acetate extract.

The quantitative estimation of ethyl acetate extract and Fraction 1 for gallic acid, catechin, ursolic acid, quercetin, berberine, rutin and piperine was carried out. The ethyl acetate extract and the Fraction 1 show only the presence of gallic acid and catechin. The amount of gallic acid present in the ethyl acetate extract was found to be 0.385 ± 0.012 % w/w (mean ± SD) and the gallic acid content in Fraction 1 cannot be quantified as the AUC is out of permitted range, however, the spectral scan of the Fraction 1 matches with that of standard, indicating the presence of gallic acid (Table 7 and Figure 18-20). The amount of catechin present in the Fraction 1 was found to be 0.317 ± 0.003 % w/w (mean ± SD) and the gallic acid content in the ethyl acetate extract cannot be quantified as the AUC is out of permitted range, however, the spectral scan of the ethyl acetate extract matches with that of standard, indicating the presence of catechin. (Table 8 and Figure 21-23).



Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %	Assigned substance
1	0.00 Rf	123.3 AU	0.01 Rf	157.7 AU	12.29%	0.05 Rf	0.4 AU	2255.3 AU	7.47%	unknown *
2	0.05 Rf	0.8 AU	0.07 Rf	49.2 AU	3.83%	0.10 Rf	38.4 AU	1053.0 AU	3.49%	unknown *
3	0.10 Rf	39.5 AU	0.12 Rf	75.9 AU	5.92%	0.16 Rf	55.8 AU	2469.1 AU	8.18%	unknown *
4	0.16 Rf	57.0 AU	0.18 Rf	167.2 AU	13.03%	0.23 Rf	94.8 AU	5209.7 AU	17.26%	unknown *
5	0.23 Rf	95.8 AU	0.26 Rf	267.0 AU	20.81%	0.32 Rf	5.4 AU	7847.3 AU	26.00%	unknown *
6	0.32 Rf	5.5 AU	0.35 Rf	16.5 AU	1.28%	0.37 Rf	0.0 AU	326.4 AU	1.08%	unknown *
7	0.39 Rf	0.1 AU	0.45 Rf	24.6 AU	1.92%	0.51 Rf	3.6 AU	995.2 AU	3.30%	unknown
8	0.57 Rf	0.7 AU	0.60 Rf	5.1 AU	0.39%	0.61 Rf	3.7 AU	77.9 AU	0.26%	unknown *
9	0.64 Rf	0.3 AU	0.68 Rf	3.5 AU	0.27%	0.74 Rf	0.0 AU	73.8 AU	0.24%	unknown *
10	0.79 Rf	0.0 AU	0.82 Rf	8.5 AU	0.66%	0.83 Rf	3.1 AU	142.8 AU	0.47%	unknown *
11	0.84 Rf	0.3 AU	0.85 Rf	1.2 AU	0.10%	0.89 Rf	0.1 AU	12.4 AU	0.04%	unknown *
12	0.90 Rf	0.1 AU	0.94 Rf	177.9 AU	13.86%	0.96 Rf	101.5 AU	3708.1 AU	12.28%	unknown *
13	0.96 Rf	105.3 AU	0.98 Rf	328.7 AU	25.62%	1.00 Rf	56.9 AU	6014.1 AU	19.92%	unknown *

Figure 16: HPTLC finger printing of ethyl acetate extract of *Actiniopteris radiata*



Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %	Assigned substance
1	0.00 Rf	1.1 AU	0.02 Rf	125.0 AU	10.02%	0.07 Rf	0.1 AU	1603.9 AU	5.77%	unknown *
2	0.08 Rf	0.1 AU	0.19 Rf	266.2 AU	21.33%	0.20 Rf	238.8 AU	8304.8 AU	29.89%	unknown *
3	0.21 Rf	230.6 AU	0.24 Rf	601.1 AU	48.17%	0.31 Rf	5.2 AU	13436.9 AU	48.36%	unknown
4	0.89 Rf	0.1 AU	0.93 Rf	70.6 AU	5.66%	0.96 Rf	18.1 AU	1373.7 AU	4.94%	unknown *
5	0.96 Rf	19.5 AU	0.98 Rf	185.1 AU	14.83%	1.00 Rf	0.0 AU	3068.5 AU	11.04%	unknown *

Figure 17: HPTLC finger printing of Fraction 1 of ethyl acetate extract

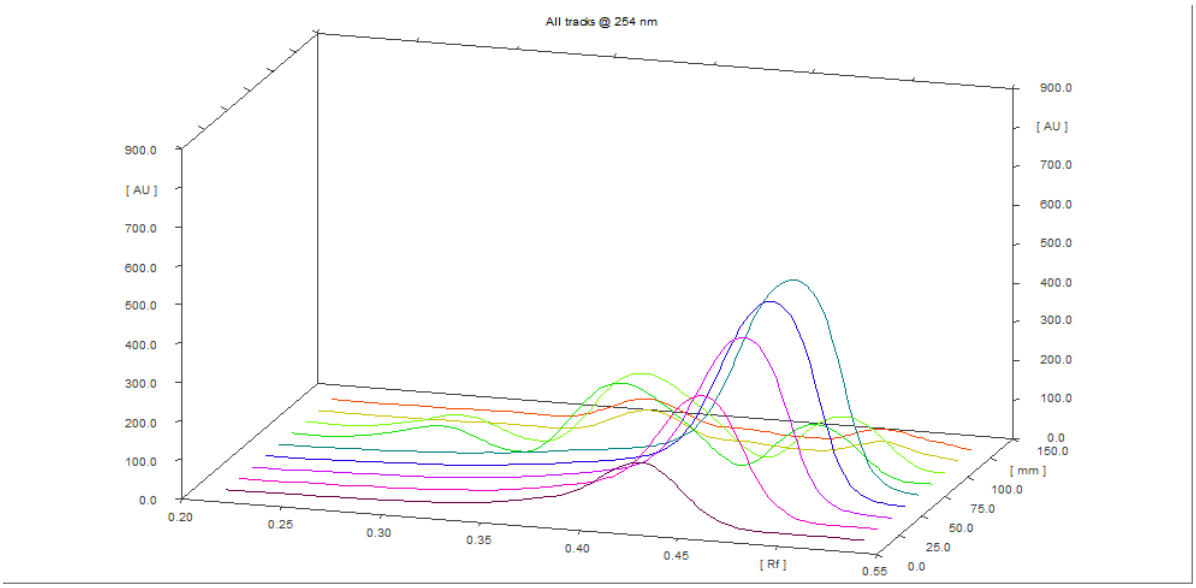


Figure 18: HPTLC densitogram comparison of gallic acid in the ethylacetate extract, Fraction 1 and the standard

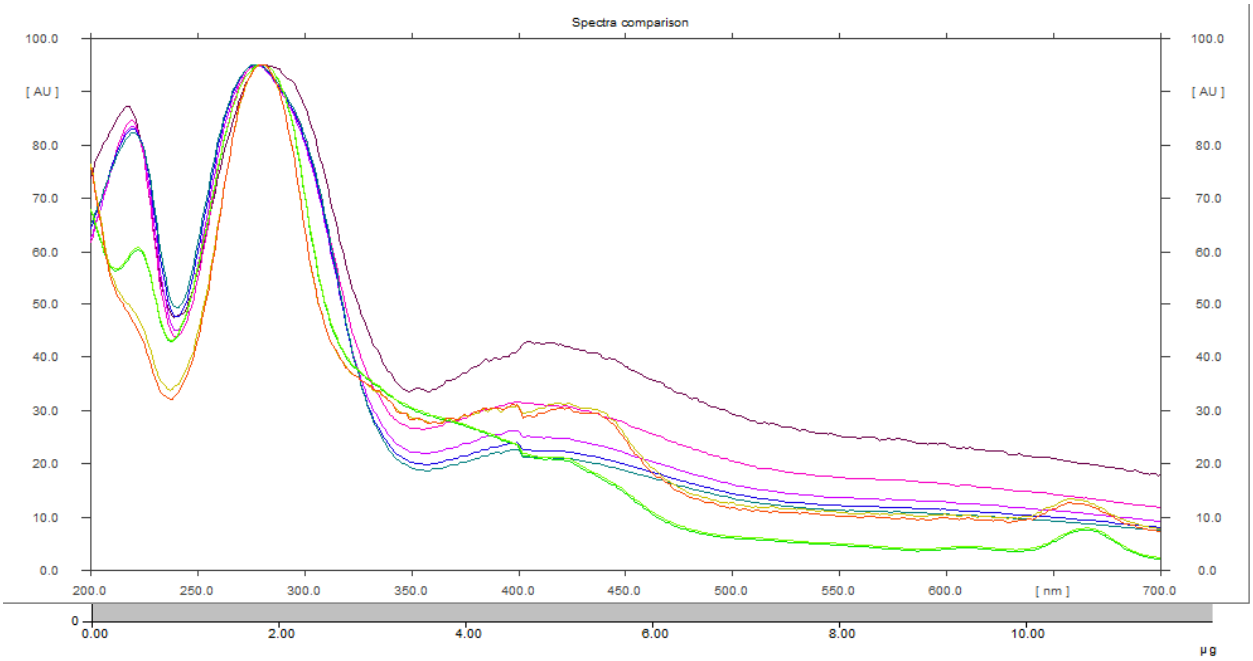


Figure 19: HPTLC spectral comparison of gallic acid in the ethylacetate extract, Fraction 1 and the standard

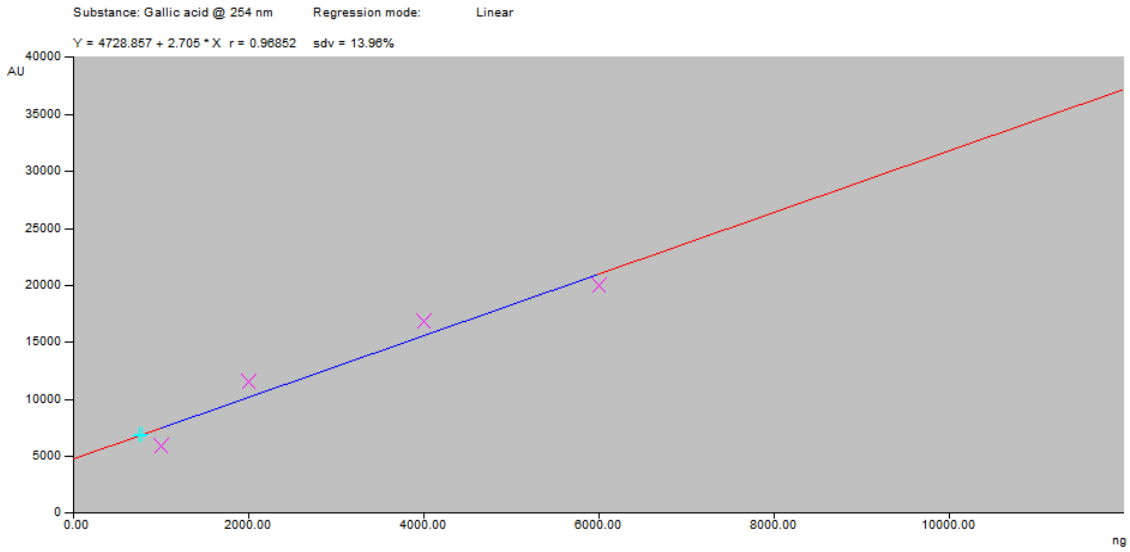


Figure 20: Linear calibration curve of gallic acid standard

Table 7: Quantitative estimation of gallic acid content in ethyl acetate extract and Fraction 1 using regression equation

Track	Vial	Rf	Amount Fraction (µg)	Area	Calculated amount (ng)	Remark	% w/w	Average % w/w (mean ± SD)
1	1	0.42	1.000	5821.09		Std Level 1		
2	1	0.45	2.000	11534.16		Std Level 2		
3	1	0.46	4.000	16785.71		Std Level 3		
4	1	0.47	6.000	19933.95		Std Level 4		
5	1					Std Level 5		
6	2	0.38		6858.04	787.25	EA extract	0.393564	0.385 ± 0.012
7	2	0.39		6764.97	752.84	EA extract	0.376361	
8	3	0.38		1764.41	<0.0 g	Fraction 1	Out of permitted range	Out of permitted range
9	3	0.37		1757.5	<0.0 g	Fraction 1		

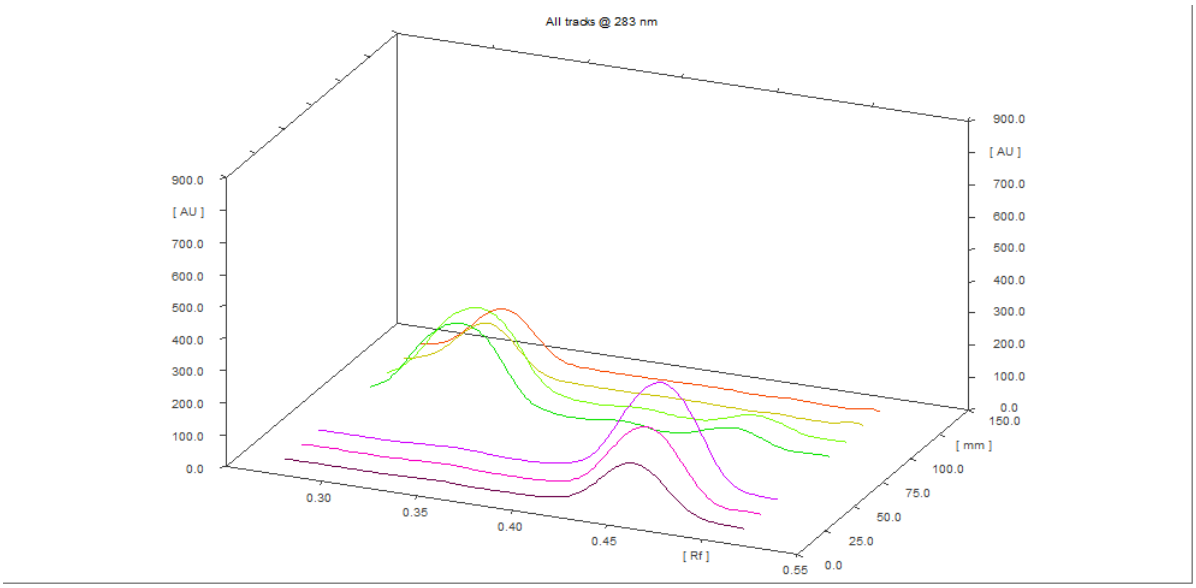


Figure 21: HPTLC densitogram comparison of catechin in the ethylacetate extract, Fraction 1 and the standard

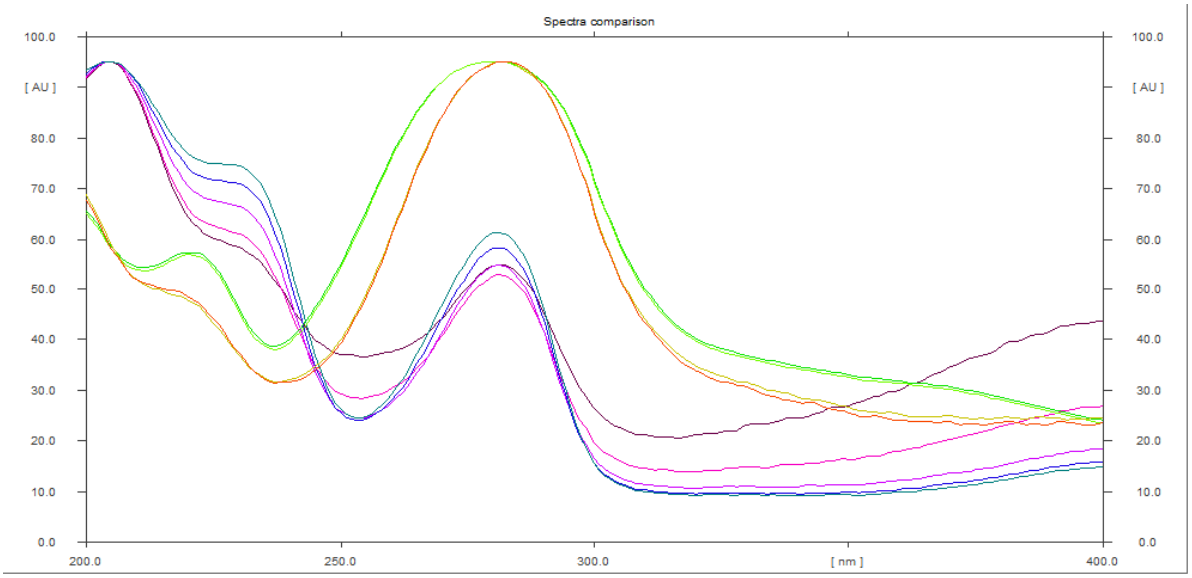


Figure 22: HPTLC spectral comparison of catechin in the ethylacetate extract, Fraction 1 and the standard

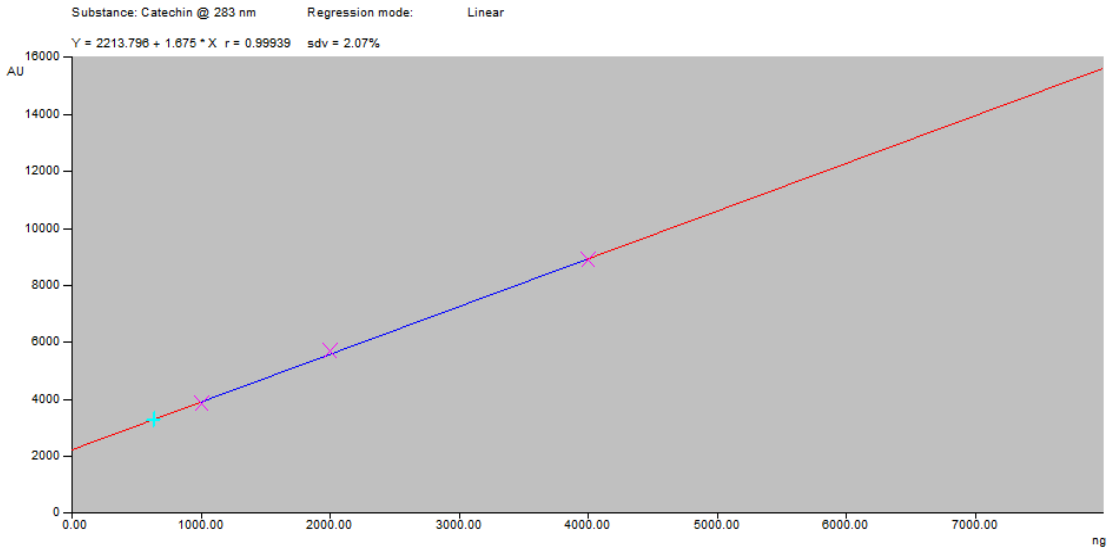


Figure 23: Linear calibration curve of catechin standard

Table 8: Quantitative estimation of catechin content in ethyl acetate extract and Fraction 1 using regression equation

Track	Vial	Rf	Amount Fraction	Area	Calculated amount (ng)	Remark	% w/w	Average % w/w (mean ± SD)
1	1	0.46	1.000 µg	3821.35		Std Level 1		
2	1	0.45	2.000 µg	5665.96		Std Level 2		
3	1	0.45	4.000 µg	8881.06		Std Level 3		
4	1					Std Level 4		
5	1					Std Level 5		
6	2	0.41		186.2	<0.0 g	EA extract	Out of permitted range	Out of permitted range
7	2	0.47		771.65	<0.0 g	EA extract		
8	3	0.32		3281.54	637.35 ng	Fraction 1	0.319	0.317 ± 0.003
9	3	0.32		3267.19	628.79 ng	Fraction 2	0.314	

5.8 *In vitro* antioxidant studies of *Actiniopteris radiata*

The petroleum ether, chloroform, ethyl acetate and ethanol extracts were screened for *in vitro* antioxidant activity. The *in vitro* antioxidant studies includes DPPH scavenging assay, ABTS scavenging assay, Nitric oxide assay, Super oxide assay and Deoxyribose assay. Among the extracts tested for *in vitro* antioxidant activity, ethanol and ethyl acetate extract exhibited potent antioxidant activity in DPPH, ABTS, Nitric oxide, Super oxide and Deoxyribose methods. The values of extracts are compared with the values of standards ascorbic acid and rutin.

5.8.1 DPPH radical scavenging assay

DPPH generated radical was tested for *Actiniopteris radiata* along with the rutin and ascorbic acid. DPPH is a stable free radical. The assay is based on the measurement of the scavenging ability of antioxidants towards stable radical $DPPH^{\cdot}$. *Actiniopteris radiata* extract reduces the radical to the corresponding hydrazine when it reacts with the hydrogen donors in the antioxidants. DPPH radicals react with suitable reducing agents, the electrons become paired off and the solution loses colour stoichiometrically depending on the number of electrons taken up. The ethanol extract of *Actiniopteris radiata* has shown potent antioxidant activity with half inhibition concentration (IC_{50}) of 1.98 ± 0.13 µg/ml.

5.8.2 ABTS radical cation scavenging assay

The ABTS assay is based on the inhibition of the absorbance of the radical cation $ABTS^{+}$. The ABTS chemistry involves direct generation of ABTS radical mono cation with no involvement of any intermediary radical. It is a decolorisation assay, thus the radical cation is performed prior to addition of antioxidant test system. The extract act either by inhibiting or scavenging the $ABTS^{+}$ radicals. The ethanol extract of *Actiniopteris radiata* has shown potent antioxidant activity with half inhibition concentration (IC_{50}) of 47.48 ± 0.91 µg/ml.

5.8.3 Superoxide anion radical scavenging assay

In Superoxide anion scavenging activity, superoxide anions damage biomolecules directly or indirectly by forming H_2O_2, $^{\cdot}OH$, peroxy nitrite or singlet oxygen during aging and pathological events. Superoxide directly initiate lipid peroxidation. The superoxide radical scavenging activity of *Actiniopteris radiata* extracts were assayed by the PMS-NADH system. The superoxide scavenging activity of *Actiniopteris radiata* extracts were increased markedly with the increase in concentrations. The ethylacetate extract has shown potent antioxidant activity with the half inhibition concentration (IC_{50}) was 18.62 ± 3.82 µg/ml. These results suggested that *Actiniopteris radiata* extract has a potent superoxide radical scavenging effects.

5.8.4 Nitric oxide radical scavenging assay

In Nitric oxide scavenging activity, nitric oxide (NO) is a free radical produced in mammalian cells, involved in the regulation of various physiological processes. The excess production of NO is associated with several diseases. Nitric oxide is very unstable species under aerobic condition. It reacts with oxygen to produce stable product nitrate and nitrite through intermediates NO_2, N_2O_4 and N_3O_4. The nitrite produced by the incubation of solution of sodium nitroprusside in standard phosphate buffer at 25^{o}C was reduced by the *Actiniopteris radiata* extract. This may be due to the antioxidant principles in the extract which compete with oxygen to react with nitric oxide and thus inhibits the generation of nitrite. The ethanol extract has potent antioxidant activity with IC_{50} value of 109.40 ± 6.06 µg/ml.

5.8.5 Deoxyribose degradation assay

In Deoxyribose method, increasing concentration of antioxidant reduces DNA expression. The control DNA exhibits both super-coiled and open circular forms. Incorporation of test compounds damages the super-coiled form and at the same time increases the expression of open circular form. The test compounds damage the super-coiled form. Further the damage exerted by H_2O_2 and $FeCl_3$ was reduced at higher concentrations of *Actiniopteris radiata* extract. The ethyl acetate extract of *Actiniopteris radiata* has shown antioxidant activity with half inhibition concentration (IC_{50}) of 144.30 ± 8.79 µg/ml. The half inhibition concentration

values of petroleum ether, chloroform, ethyl acetate and ethanol extracts of *Actiniopteris radiata* has been given in **Table 9**.

Table 9. *In vitro* antioxidant activity of *Actiniopteris radiata* extracts

Extract	IC_{50} values ± S.E.M (µg/ml)				
	DPPH	ABTS	Nitric oxide	Super oxide	Deoxy ribose
Petroleum ether	>1000.00	>1000.00	>1000.00	>1000.00	>1000.00
Chloroform	386.60 ± 5.31	658.00 ± 3.56	880.54 ± 4.05	223.18 ± 1.44	286.13 ± 1.83
Ethyl acetate	8.55 ± 0.42	111.50 ± 4.29	390.50 ± 11.05	18.62 ± 3.82	144.30 ± 8.79
Ethanol	1.98 ± 0.13	47.48 ± 0.91	109.40 ± 6.06	330.50 ± 1.53	186.03 ± 2.03
Standards					
Ascorbic acid	4.83 ± 0.38	11.32 ± 0.28	-----	------	------
Rutin	7.82 ± 0.16	9.39 ± 0.59	84.23 ± 2.54	------	74.63 ± 1.62

In Vitro Alpha glucosidase inhibition activity

The results of the *in vitro α–glucosidase* inhibition activity of petroleum ether, chloroform, ethyl acetate and ethanol extracts and fractions of ethyl acetate extract are given in **Table 10**. Among the samples tested only ethyl acetate extract showed good inhibition activity (IC_{50}73.25 ± 0.7 µg/ml) and the results were comparable to standard, acarbose (IC_{50} 38 05 ± 0.3 µg/ml). The fractions F5, F6 and F7 shown only mild activity.

5.10 *In Vivo* Antidiabetic activity

All the glucose lowering agents available today for treatment of diabetes resulted from *in vivo* anti-diabetic drug discovery approach. The ethyl acetate extract has significant activity in alpha glucosidase inhibition method. Hence ethyl acetate extract was selected for *in vivo* anti-diabetic screening.

5.10.1 Oral glucose tolerance test

The oral glucose tolerance test was performed in overnight (18-h) fasted normal rats. The rats were divided into 5 groups with 5 rats in each group. Group 1 – glucose control, Group 2 – Glibenclamide(10mg/kg), Group 3 – petroleum ether extract(100mg/kg), Group 4 –

chloroform extract(100mg/kg), Group 5 – ethyl acetate extract(100mg/kg) and Group 6 – ethanol extract(100 mg/kg). Zero hour blood glucose was determined in overnight fasted rats. After 30 min of drug treatment, the rats were fed with 2g/kg glucose and blood glucose was determined after 30, 60, 120 and 180 min of the glucose load [107]. Blood glucose concentration was estimated by GOD – POD method. The ethanol and ethyl acetate extract were shown significant antihyperglycemic activity. The values are given in **Table 11**.

Table 10: In vitro alpha-glucosidase inhibition activity of extracts of Actiniopteris radiata

Name of the sample	**IC_{50} (µg/ml)**
Pet. ether	Not active
Chloroform	Not active
Ethyl acetate	73.25 ± 0.7
Ethanol extract	Not active
Acarbose	38.05 ± 0.3
Fraction 1	Not active
Fraction 2	Not active
Fraction 3	Not active
Fraction 4	Not active
Fraction 5	573.85 ± 5.7
Fraction 6	773.23 ± 8.5
Fraction 7	873.11 ± 9.2
Fraction 8	Not active
Fraction 9	Not active
Fraction 10	Not active
Fraction 11	Not active
Fraction 12	Not active
Fraction 13	Not active
Fraction 14	Not active
Fraction15	Not active

Values are mean ± SD, n=3

Table 11, Effect of the extracts in glucose loaded hyperglycemic rats.

Group	Treatments	Blood glucose concentration (mg/dl)				
		0 min	30 min	60 min	120 min	180 min
1	Glucose control	78.28 ± 1.99	148.12 ± 2.30	157.10 ± 3.88	127.31 ± 3.30	102.30 ± 4.52
2	Glibenclamide (10mg/kg)	80.24 ± 2.22	108.15 ± 1.62**	93.17 ± 1.17**	76.21 ± 1.95**	69.29 ± 3.94**
3	Pet. ether extract (100 mg/kg)	82.11 ± 2.18	116.31 ± 2.58*	108.40 ± 0.46**	95.88 ± 0.72**	87.20 ± 0.91**
4	Chloroform extract (100 mg/kg)	80.64 ± 0.98	141.34 ± 1.32**	126.32 ± 2.38**	118.52 ± 1.62**	97.46 ± 0.68**

5	Ethyl acetate extract (100 mg/kg)	84.04 ± 2.07	128.20 ± 0.64*	117.28 ± 0.35**	94.25 ± 0.56**	86.24 ± 0.77**
6	Ethanol extract (100 mg/kg)	82.21 ± 1.73	124.32 ± 0.82*	118.45 ± 1.20**	90.25 ± 0.71**	74.04 ± 1.40**

Each value represents the mean ± S.E.M of five observations. *P<0.05, **P<0.001 vs glucose control (one way ANOVA followed by Tukey's Multiple Comparison Test)

5.10.2 Estimation of biochemical parameters

Blood was withdrawn from the retroorbital sinus under ether anaesthesia. The serum was separated immediately by centrifugation. The serum was analysed for glucose, cholesterol, triglyceride, HDL cholesterol and LDL cholesterol using biochemical kits [108, 109]. The values are given in **Table 12**.

Table 12, Effect of extracts on biochemical parameters.

Animal group	S.glucose mg/dl	S.cholesterol mg/dl	S.triglyceride mg/dl	S.HDL mg/dl	S.LDL mg/dl
Normal control	82.10 ± 3.62**	65.30 ± 3.10**	63.15 ± 3.46**	52.13 ± 2.60**	23.01 ± 0.48**
Diabetic control	503.18 ± 6.31	86.23 ± 4.31	121.53 ± 3.68	38.71 ± 1.30	33.47 ± 2.36
Ethyl acetate extract 100mg/kg b.w	77.98 ± 1.62**	56.11 ± 1.42**	93.59 ± 3.32**	45.31 ± 0.86**	28.63 ± 0.82**
Ethyl acetate extract 200mg/kg b.w	72.92 ± 1.15**	51.48 ± 2.19**	53.06 ± 0.74**	51.80 ± 1.07**	29.62 ± 0.74**
Ethyl acetate extract 400mg/kg b.w	68.33 ± 1.46**	59.53 ± 1.41**	58.69 ± 1.70**	48.92 ± 0.88**	28.07 ± 1.01**
Glibenclamide 10mg/kg b.w	76.31± 0.828**	61.32 ± 3.20**	61.21± 2.30**	46.18 ± 0.72**	22.31 ± 2.51**

Each value represents the mean ± S.E.M of five observations. **P<0.001 vs diabetic control (one way ANOVA followed by Tukey's Multiple Comparison test)

Statistical analysis

The values are expressed as mean ± SEM. The results were analysed for statistical significance using one-way ANOVA, followed by Tukey's Multiple Comparison test. P<0.05 was considered significant.

6. DISCUSSION

The present study was undertaken to examine the antidiabetic activity of *Actiniopteris radiata*. The effect of extract in diabetes changes in associated complications, biochemical parameters was also assessed. Wistar rats of either sex were induced diabetic by streptozotocin. The blood glucose level above 200 mg/dl were considered diabetic and used for this experiment. The diabetic rats were treated for 7 days with ethyl acetate extract and glibenclamide. Biochemical parameters were estimated after treatment.

In the glucose loaded hyperglycemic model, the extracts were tested for antihyperglycemic activity, ethanol and ethyl acetate extracts were exhibited significant antihyperglycemic activity at a dose level of 100 mg/kg. Chloroform extract has least antihyperglycemic activity at a dose level of 100 mg/kg. Excessive amount of glucose in the blood induces the insulin secretion. This secreted insulin will stimulate peripheral glucose consumption and control the production of glucose through different mechanisms [110]. However, from the study (glucose control), it was clear that the secreted insulin requires 2-3 h to bring back the glucose level to normal.

In general, an increase in blood glucose level is usually accompanied by an increase in plasma cholesterol, triglyceride, LDL levels and a decrease in HDL levels as observed in diabetic rats [111]. The marked hyperlipidemia (increase in the level of lipid in the body) that characterizes the diabetic state may be the consequence of the uninhibited actions of lipolytic hormones on fat depots [112]. The ethyl acetate extract at a dose of 400 mg/kg b.w has significant activity than lower dose of ethyl acetate extract (100 mg/kg b.w). The significant reduction in the blood glucose level comparable to that produced by glibenclamide treatment [113]. Further ethyl acetate extract was purified by column chromatography that led to isolation of a two new compounds [114]. The flavones are present in the ethyl acetate extract, it resulted in a decrease in plasma glucose and increase in insulin levels. The flavones also mimics the effects of insulin [115].

In conclusion, this study has shown that ethyl acetate extract of *Actiniopteris radiata* has significant antidiabetic activity. This research supports the inclusion of this plant in antidiabetic preparations and useful in development of antidiabetic drug.

7. SUMMARY AND CONCLUSION

- The traditional herbal medications are become mainstream throughout the world. Since ancient times, plants have been source of medicines. Ayurveda and other Indian literature mention the use of plants in treatment of various human diseases. India has about 45000 plant species and among them several thousands have medicinal properties. Plants are major source of drugs and many of the currently available drugs have been derived directly or indirectly from them.

- Since ancient times, plants have been used in the treatment of diabetes mellitus. There are many hypoglycemic plants and their active principles varies. Therefore considerable diversity in the mechanism of action. Some act by increasing the release of insulin and require a minimum of β-cells to exert their action. Other plant extracts or constituents act by modifying glucose metabolism. All are important since they are used to treat the different aspects of diabetes mellitus.

- The selected plant *Actiniopteris radiata* was collected from Nilgiri district, Tamilnadu and authenticated. The dried plant material was subjected to successive extraction with petroleum ether, chloroform, ethyl acetate and ethanol by soxhlet method. The extracts were concentrated under reduced pressure and controlled temperature.

- The phytochemical studies of the extracts gave positive test for the flavanoids, carbohydrates, hydrocarbons, fatty acids, sterols, steroids, steroidal glycoside and unknown glycoside. Determination of water soluble extract, alcohol soluble extract, total ash, acid insoluble ash and water soluble ash were carried out. The quantitative phytochemical estimations total phenol content and total flavonoid content of the extracts were estimated.

- Qualitative and quantitative determinations of ethyl acetate extract and fractions were done by HPTLC method.
- *In vitro* antioxidant studies DPPH, ABTS, Nitric oxide, Super oxide and Deoxy ribose were carried out. The ethyl acetate extract shown potent *in* vitro antioxidant activity. The ethyl acetate extract of *Actiniopteris radiata* was selected for *in vivo* anti-diabetic activity based on the in *vitro* antioxidant activity.

- *In vitro* antidiabetic activity was performed by *alpha glucosidase* inhibition activity. The successive extracts of *Actiniopteris radiata* and fractions of ethyl acetate extract were screened for *alpha glucosidase* inhibition activity. The results was compared with the values of standard (acarbose). The ethyl acetate extract has shown significant antidiabetic activity and fraction 5, 6, 7 shown moderate antidiabetic activity. The ethyl acetate extract was selected for *in vivo* antidiabetic activity based on the results of *in vitro* antidiabetic activity.

- Wistar rats of either sex weighing 180-220 g (6 to 8 weeks) with no prior drug treatment were used for *in vivo* anti-diabetic activity. The acute toxicity study of the ethyl acetate extract was determined according to the O E C D guidelines No.425. Female Wistar rats weighing 180-220 g (6 to 8 weeks) were used for this study. The test samples in a single dose of 400 mg/kg b.w, 800 mg/kg b.w and 2 g/kg b.w were given orally. The animals were observed for 24 hours and monitored for 14 days to record general behaviour and mortality. No mortality was observed till the end of the study.

- Streptozotocin was used to induce diabetes. Streptozotocin 55 mg/kg b.w was administered intraperitoneally. The rats with blood glucose level above 200 mg/dl were considered diabetic and used in the experiment.

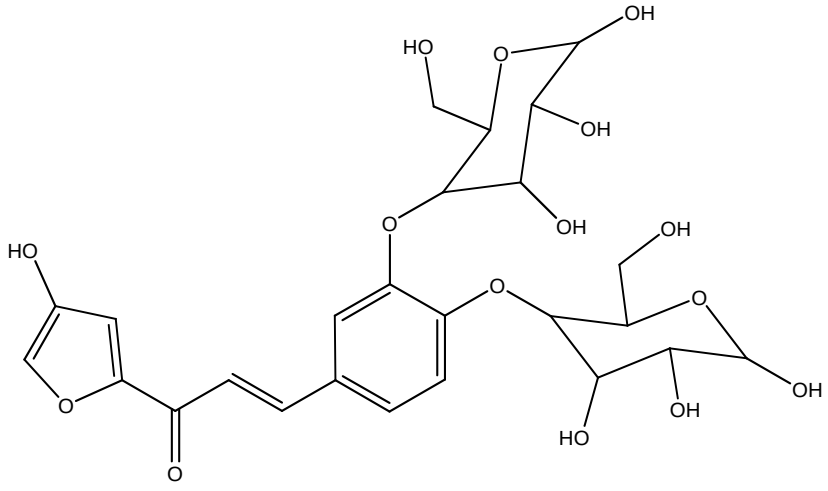
- The oral glucose tolerance test was performed in overnight fasted normal rats. Zero hour blood sugar was determined in overnight fasted rats. After 30 min of drug treatment, the rats were fed with 2 g/kg b.w glucose and blood glucose was determined after 30, 60, 120

and 180 min of the glucose load. Blood glucose concentration was estimated by GOD-POD method. The ethanol and ethyl acetate extract have shown significant antidiabetic activity.

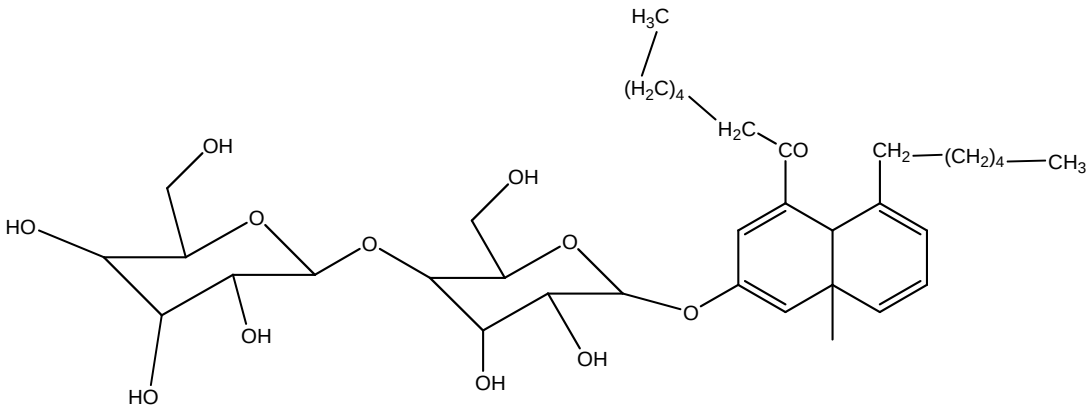
- Blood was withdrawn from the retroorbital sinus under ether anaesthesia. The serum was separated immediately by centrifugation and analysed for glucose, cholesterol, triglyceride, HDL cholesterol and LDL cholesterol.

- The ethyl acetate extract was selected for *in vivo* anti-diabetic activity. The ethyl acetate extract was administered at a dose of 100, 200 and 400 mg/kg b.w for 7 days. The ethyl acetate extract treatment reduces the glucose, triglycerides, cholesterol, LDL cholesterol and increases the HDL cholesterol. The ethyl acetate extract at 400 mg/kg b.w shown significant antidiabetic activity. The results are compared with the standard.

- The ethyl acetate extract was subjected to column chromatography and isolated the active constituents. Two new compounds were isolated and characterised by TLC, IR, UV spectral analysis, NMR and Mass spectra. Compound 1 is 2-(3, 4-O – Diglucos cinnamoyl) – 4 – hydroxyl furan and compound 2 is 1-Heptaloyl, 8-hexyl, 3-(O – diglucos), 10 – methyl, 9. 10 – dihydro naphthalene.



2-(3, 4-O–Diglucos cinnamoyl) – 4 – hydroxyl furan



1-Heptaloyl, 8-hexyl, 3-(O–diglucos), 10 – methyl, 9. 10 – dihydro naphthalene.

Objectives achieved

1. The potent *in vitro* antioxidant activity was found in the ethyl acetate extract of *Actiniopteris radiata*.
2. The significant antidiabetic activity of ethyl acetate extract was found in *alpha glucosidase* inhibition activity.
3. The potent *in vivo* antidiabetic activity was found in the ethyl acetate extract of *Actiniopteris radiata*.
4. Two new compounds were isolated and characterised by TLC, IR, UV spectral analysis, NMR and Mass spectra. Compound 1 is 2-(3, 4-O–Diglucos cinnamoyl) – 4 – hydroxyl furan and compound 2 is 1-Heptaloyl, 8-hexyl, 3-(O–diglucos), 10 – methyl, 9. 10– dihydro naphthalene.
5. The *in vitro* antidiabetic, *in vitro* antioxidant and *in vivo* antidiabetic activities were performed for the first time for *Actiniopteris radiata*.

Scope for further research

There is scope for further research in isolating the other phytocnstituents and to carry out the other biological activities of the extract and its phytoconstituents. There is need to establish the mechanism of action of other biological activities.

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APPENDIX 1: ETHICAL COMMITTEE CERTIFICATE

J.S.S. College of Pharmacy, Ootacamund, Tamil Nadu, India.
Committee for the Purpose of control and Supervision of Experiments on Animals (CPCSEA)
Institutional Animal Ethics committee (IAEC)

CERTIFICATE

Title of the Project: Anti diabetic Screening and Phytochemical investigation of Selected Medicinal plants

Proposal Number: JSSCP /IAEC/~~NOV/PHAR~~ PH.D/ PH.CHEM/03/ 2009 - 2010

Date received after modification (if any):
Date received after second modification: 29.09.09

Approval date: 30.09.09

Animals: Wistar rats/ Albino mice
Rabbits / Guinea pigs

No. of animals sanctioned: 42

Male/Female

Expiry date (Termination of the Project): 2 MONTHS.

Name of IAEC/CPCSEA chairperson: **Dr.K.Elango**

Signature of Chairperson
Date:

Chairperson
Institutional Animal Ethics Committee
JSS College of Pharmacy
Rocklands, Ooty-643 001