3.1 Introduction:

Diabetes is a metabolic disorder which is considered as a major cause of high economic loss which can in turn impede the development of nations[1]. In order to prevent this alarming health problem, the development of research into new hyperglycaemic and potentially antidiabetic agents is of great interest and urgency. Medicinal plants play an important role in the management of diabetes mellitus especially in developing countries where resources are meagre. Owing to the requirementsfor life long continuous medication, patients - especially from the rural areas of India, prefer to use only natural remedies to control their blood glucose levels. Therefore, it is necessary to look for new solutions to manage this health problem.

Nature has been a source of medicinal treatment for many diseases for thousands of years. Plant based systems continue to play an essential role in the health care of 80% of the world's underdeveloped and developing countries[2]. India is a country with a vast reserve of natural resources and a rich history of traditional medicine. According to the World Health Organization (WHO), about 65% of the population in rural India use *Ayurveda* and medicinal plants for primary heath care[3] . Indian herbs are used as drugs and remedies to cure and mitigate diseases. Global demand for herbal products has steadily increased during the last two decades. Consequently, there has been an extensive research on the isolation and characterization of compounds from medicinal plants. Natural products continue to provide greater structural diversity than standard combinatorial chemistry and offer major opportunities for finding novel molecules that are active against a wide range of assay targets. Active compounds, to which the medicinal properties are attributed, need to be isolated and their structures should be established.

3.2 Plants used for screening on the basis of traditional knowledge

Twenty different plants were collected from the various places of Tinsukia, Dibrugarh and Sonitpur districts of Assam, India on the basis of their use in the treatment of diabetes by various ethnic groups inhabiting these areas. Taxonomic classification and a brief description of the used 20 plants are as follows:

3.2.1 *Trapa natans* L.

Source: cfb.unh.edu

Kingdom	**:**	Plantae
Phylum	**:**	Tracheophyta
Class	**:**	Magnoliopsida
Order	**:**	Myrtales
Family	**:**	Lythraceae
Genus	**:**	*Trapa*
Species	**:**	*Trapa natans* L.

.

3.2.1.1Brief description of the plant

Trapa natans L.is an annual floating-leaved plant, growing in stagnant waters bodies up to 5 m deep, native to warm temperate parts of Eurasia and Africa. The floating leaves are arranged in a rosette, with leathery dorsal surface. These are broadly rhomboid, triangular, deltoid or broadly ovate[4]. The leaves are sharply serrate, with conspicuous venation and short, stiff hairs. The plant has solitary white flowers with four 8 mm-long petals and four green sepals.The fruit is hard, woody, horned nut-like structure that develops underwater[5].

3.2.1.2 Medicinal use of the species

It is used in cases of sexual debility, spermatorrhea, general debility, fatigue, tuberculosis, intermittent fevers [6] .Fruits are refrigerant and useful in dysentery, dry cough bilious affections. The fruits are also used as intestinal astringent, aphrodisiac, and antiinflammatory, and in leprosy, urinary discharges, fractures, sore throat, and anaemia[7].

3.2.2 *Terminalia chebula* Retz.

Source: www.onlyfoods.net

Kingdom	**:**	Plantae
Phylum	**:**	Tracheophyta
Class	**:**	Magnoliopsida
Order	**:**	Myrtales
Family	**:**	Combretaceae
Genus	**:**	*Terminalia*
Species	**:**	*Terminalia chebula* Retz.

3.2.2.1 Brief description of the plant

Terminalia chebula Retz. is commonly known as yellow- or chebulic myrobalan and is native to South Asia. It is a deciduous tree of height 50-80 ft. The leaves are ovate and elliptical that is alternately arranged. Flowers are yellowish-white;unpleasantly scented with an axillary or terminal, simple or branched spike *i*nflorescence. Fruit is obovoid or cylindrical-ellipsoid drupe that is 2.5–5 cm long, yellow to orange-brown and glabrous[8].

3.2.2.2 Medicinal use of the species

T. chebula Retz. exhibits many medicinal activities due to the presence of diverse phytoconstituents. The fruit of *T. chebula* Retz.is used for the treatment of digestive diseases, urinary diseases, diabetes, skin diseases, heart diseases, irregular fevers, constipation, ulcers, vomiting, colic pain, haemorrhoids[9].

3.2.3 *Cinnamomum tamala* (Buch.-Ham.) T. Nees&Eberm.

Kingdom	**:**	Plantae
Phylum	**:**	Tracheophyta
Class	**:**	Magnoliopsida
Order	**:**	Laurales
Family	**:**	Lauraceae
Genus	**:**	*Cinnamomum*
Species	**:**	*Cinnamomum tamala* (Buch.-Ham.) T. Nees&Eberm.

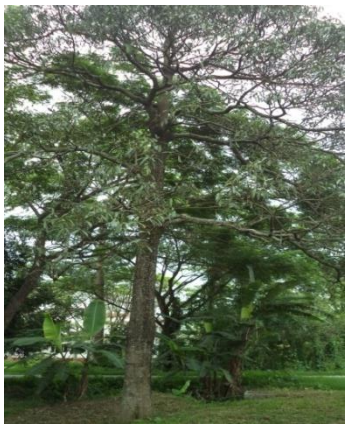
3.2.3.1Brief description of the plant

Cinnamomum tamala (Buch.-Ham.) T. Nees&Eberm. is an evergreen tree growing upto 10-20 m tall. Its habitat includes mainly the mountain slopes, evergreen broad-leaved forests in valleys and watersides at elevations of 1,100 - 2,000 m. Leaves are lanceolate, glabrous; alternate, opposite and short stalked. Inflorescence is characterised by a terminal or axillary hairy panicle bearing yellow flowers. Fruits are berry like and ovoid.

3.2.3.2Medicinal use of the plant:

The dried bark is used to treat stomach-ache.The leaves are used in the treatment of colic and diarrhoea. The leaf extracts produce a hypoglycaemic effect in experimental rats. The hydrodistilled essential oils of *C. tamala* have their anti-fungal activity against *Trichophyton mentagrophytes* and *Microsporum microsporumaudounil*, which cause ring worm diseases in animals and human. Plant parts are used in many *ayurvedic* preparations, e.g., *sudarshan*, *choorna* and *chanderprabhavati*[10].

3.2.4 *Alstonia scholaris* (L.) R. Br.



Kingdom	**:**	Plantae
Phylum	**:**	Tracheophyta
Class	**:**	Magnoliopsida
Order	**:**	Gentianales
Family	**:**	Apocynaceae
Genus	**:**	*Alstonia*
Species	**:**	*Alstonia scholaris* (L.) R. Br.

3.2.4.1 Brief description of the plant

Alstonia scholaris (L.) R. Br., commonly known as the devil tree is an evergreen tropical tree native to India and the sub-continent. It is a glabrous tree that grows upto 40m. Its mature bark looks grayish in colour and its leaves occur in

whorls of 3-10 which are leathery, narrowly obovate or spathulate[11]. Flowers are small, greenish white, many in umbellate panicles, corolla tube is short, very strongly scented and exhibit a much-branched terminal panicle inflorescence. Fruits occur in thin pods which can be upto 20inches long[12].

3.2.4.2 Medicinal use of the species

The ripe fruits of the plant are used in syphilis and epilepsy. The milky juice of *A. scholaris* (L.) R. Br.has been applied to treat ulcers.The bark of the *A.scholaris* (L.) R. Br.is used in Ayurvedic medicine to treat fever, malariaand troubles in digestion, tumors, ulcers, asthma, and so forth. The leaves and the latex are applied externally to treat tumors. The roots and barkare used in traditional medicine as an anthelmintic, astringent tonic, alterative, antidiarrhoeaticum, antiperiodicum, etc[13].

3.2.5*Cassia alata* L.

Kingdom :		Plantae
Division	**:**	Magnoliophyta
Class	**:**	Magnoliopsida
Order	**:**	Fabales
Family	**:**	Leguminosae
Genus	**:**	*Cassia*
Species	**:**	*Cassia alata* L.

3.2.5.1 Brief description of the plant

Cassia alata L. commonly known as the Candle Sticks or Candle Bush is a shrub that generally grows upto 3 m in height with leaves 50-80cm in length. It is perennial in nature and grows well in tropical climate. The leaves are simple pinnate. The flowers are yellow in colour exhibiting racemose or cymose inflorescense. The fruits are pods with two broad wings with square shaped seeds[13].

3.2.5.2 Medicinal use of the species

The leaves or sap is used in the treatment of fungal infections like ringworm, etc. It is also used to cure high blood pressure, asthma, stomach problems and several skin diseases[14].

3.2.6 *Nyctanthes arbor-tristis* L.

Kingdom : Plantae
Phylum : Tracheophyta
Class : Magnoliopsida
Order : Lamiales
Family : Oleaceae
Genus : *Nyctanthes*
Species : *Nyctanthes arbor-tristis* L.

3.2.6.1 Brief description about the plant

Nyctanthes arbor-tristis L., popularly known as 'Tree of Sorrow', is an indigenous small tree, with a gray or greenish, rough and flaky bark. It grows to a height of 10m approximately. This shrub consists of simple opposite leaves; the flowers are fragrant with a five-to-eight lobed corolla and orange-red centre, often in cymose inflorescence. The fruit is flat, brown and heart-shaped to rounded-capsule. It is native to Southern Asia, stretching across Northern Pakistan and Nepal through Northern India to Southeast Thailand[14].

3.2.6.2 Medicinal use of thespecies

N. arbor-tristis L. also known as Coral Jasmine has antibacterial, anti-inflammatory, anthelmintic property. It is used to treat sciatica, arthritis, fevers, rheumatism and constipation in children. It is also used in the treatment of fungal skin infection, dry cough, and bronchitis and as an antidote for snakebites[15].

3.2.7 *Phlogacanthus thyrsiflorus* Nees.

Source: biodiversity.bt

Kingdom	**:**	Plantae
Phylum	**:**	Tracheophyta
Class	**:**	Magnoliopsida
Order	**:**	Lamiales
Family	**:**	Acanthaceae
Genus	**:**	*Phlogacanthus*
Species	**:**	*Phlogacanthus thyrsiflorus* Nees

3.2.7.1 Brief description of the plant

It is an evergreen shrub that grows upto 2.4m height with quadrangular branchlets. Leaves are oblanceolate, elliptic, oblong, acute or acuminate, entire. Flowers are in terminal elongated, thyrsoid panicles. In early spring the plant becomes showy with its dense cylindrical spikes of brick red velvety flower which remains in racemose inflorescence[16].

3.2.7.2 Medicinal use of the species

It is useful for curing coughs, colds and asthma. The flowers of *P. thyrsiflorus* Nees are used as antidote to pox. It prevents skin disease like sore, scabies, etc. The leaves extracts of *P. thyrsiflorus* Nees treat diarrhoea and produce significant reductions in fecal output and frequency of droppings. It also has antibacterial property and acts as an antioxidant[17].

3.2.8 *Potentilla anserine* L.

Source: www.tipdisease.com

Kingdom	**:**	Plantae
Phylum	**:**	Magnoliophyta
Class	**:**	Magnoliopsida
Order	**:**	Rosales
Family	**:**	Rosaceae
Genus	**:**	*Potentilla*
Species	**:**	*Potentilla anserina* L.

3.2.8.1Brief description of the plant

Potentilla anserine L., commonly known as the Silverweed is a low growing herbaceous plant which grows often in sandy or gravelly soil.It spreads rapidly with its stolon. The leaves are glabrous, ovate and pinnate. The flowers are buttercup-like, yellow coloured which are borne solitarily on long footstalks from the axils of the leaves[17].

3.2.8.2 Medicinal use of the species

The weed is useful in the treatment of jaundice, tetanus, etc. It was also formerly used in curing smallpox. The decoction of this weed is used for the treatment of ulcer of mouth and for fixing of loose teeth. It also used for toothache and helps in preserving gums from scurvy[18].

3.2.9 *Leucas aspera* (Willd.) Link

Kingdom	**:**	Plantae
Phylum	**:**	Tracheophyta
Class	**:**	Magnoliopsida
Order	**:**	Lamiales
Family	**:**	Lamiaceae
Genus	**:**	*Leucas*
Species	**:**	*Leucas aspera* (Willd.) Link

3.2.9.1 Brief description of the plant

Leucas aspera (Willd.) Link is an annual, branched, herb growing to a height of 15-60 cm with stout and hispid, acutely quadrangular stem and branches. Leaves are sub-sessile or shortly petiolate, linear or linearly lanceolate, obtuse, pubescent. Flowers are white, sessile, in dense terminal or axillary whorls exhibiting verticillate inflorescence. Fruits are nutlets, oblong, brown, smooth with angular inner face and rounded outer face[19].

3.2.9.2 Medicinal use of the species

L. aspera (Willd.) Link has antifungal, prostaglandin inhibitory, antioxidant, antimicrobial andantinociceptive activities[20]. It also has the ability of reducing fever. It can be inhaled to help treat nasal congestion, coughing, cold, headache,etc. The leaves are also used traditionally for insect bites and skin diseases.

3.2.10 *Bryophyllum pinnatum* (Lam.) Oken

Kingdom : Plantae
Phylum : Tracheophyta
Class : Magnoliopsida
Order : Saxifragales
Family : Crassulaceae
Genus : *Bryophyllum*
Species : *Bryophyllum pinnatum* (Lam.) Oken

3.2.10.1 Brief description of the plant

Bryophyllum pinnatum (Lam.) Oken or *Kalanchoe pinnata* is a succulent perennial, smooth, robust plant with tall hollow stems. It is commonly known as 'Air Plant' and is native to Madagascar. The herb has fleshy and leathery leaves that are opposite and decussate, egg-shaped with cordate base and pinnate upper leaflets. The margin is notched with blunt or rounded teeth from where profusion

of miniature plantlets occurs. The flowers are bell-like, pendulous, and yellowish-green tinged with pink or reddish. The inflorescence is paniculate with slender pedicel. The seeds are ellipsoid-oblong, obscurely longitudinally striate and brown[20].

3.2.10.2 Medicinal use of the species

This plant has been extensively used in treating various diseases like asthma, ulcers, urinary disorders, menstrual disorders, dysentery, diabetes, tuberculosis, etc. Its leaves are also used in mending broken bones and other factures, bruises, insect bites, etc[21].

3.2.11 *Mimosa pudica* L.

Kingdom	**:**	Plantae
Phylum	**:**	Tracheophyta
Class	**:**	Magnoliopsida
Order	**:**	Fabales
Family	**:**	Leguminosae
Genus	**:**	*Mimosa*
Species	**:**	*Mimosa pudica* L.

3.2.11.1 Brief description of the plant

Mimosa pudica L. is an annual or perennial creeping herb. It is a pantropical weed actually native to South and Central America but now it is widespread and can be found in many Asian countries. The leaves of the herb are bipinnately compound with one or two pinnae pairs, and 10–26 leaflets per pinna. The petioles are also prickly. Pedunculate pale pink or purple flower heads arise from the leaf axils with globose to ovoid heads.They are characterised by head and capitulum inflorescence. The fruits occur as pods[21].

3.2.11.2 Medicinal use of the species

Different parts of *M. Pudica* L. have been used to treat various ailments including alopecia, diarrhoea, dysentery, insomnia, tumor, and various urogenital

infections traditionally. It also has medicinal properties like antiasthmatic, aphrodisiac, analgesic, and antidepressant properties[22].

3.2.12 *Scoparia dulcis* L.

Kingdom : Plantae
Phylum : Tracheophyta
Class : Magnoliopsida
Order : Lamiales
Family : Plantaginaceae
Genus : *Scoparia*
Species : *Scoparia dulcis* L.

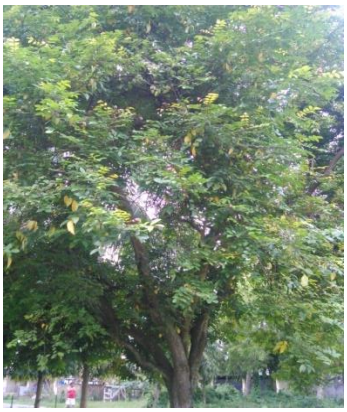
3.2.12.1 Brief description of the plant

Scoparia dulcis L. is an annual erect herb, distributed throughout tropical and subtropical regions of India, America, Brazil, West Indies, and Myanmar. Commonly known as the Sweet Broomweed, it is a small glabrous or pubescent herb with smooth or lenticellate branches. Leaves are elliptic to oblong or obovate bearing purplish black berrylike fruit.Flowers are many, in terminal panicles and are pedicelate and solitary.

3.2.12.2Medicinal use of the species

It is traditionally used in the treatment of diabetes, dysentery, earache, fever, gonorrhoea, headaches, jaundice, snake bite, stomach problems, toothache, warts. It also has antioxidant activity[23].

3.2.13 *Averrhoa carambola* L.



Kingdom	**:**	Plantae
Phylum	**:**	Tracheophyta
Class	**:**	Magnoliopsida
Order	**:**	Oxalidales
Family	**:**	Oxalidaceae
Genus	**:**	*Averrhoa*
Species	**:**	*Averrhoa carambola* L.

3.2.13.1 Brief description of the plant

Averrhoa carambola L. is a short-trunked, much branched, bushy tree that grows upto 20 to 30 ft in height. The plant originated in Indonesia and Malaysia. The leaves are deciduous, spirally arranged, imparipinnate, ovate or ovate-oblong, glabrous ventrally and pubescent dorsally. The purplish flowers grow in panicle inflorescence on red stalks. The fruit is longitudinally 5 to 6 angled, oblong which has a thin, waxy, orange-yellow skin and juicy, crisp, yellow flesh when fully ripe[24,25].

3.2.13.2 Medicinal use of the species

The fruit is used in the treatment of hemorrhages, diarrhoea, kidney and bladder complaints, etc. The seeds serve as a sedative in case of asthma and colic. It can improve the functioning of the cardiovascular system andhas antioxidant property[26].

3.2.14 *Lowsonia inermis* L.

Kingdom : Plantae
Phylum : Tracheophyta
Class : Magnoliopsida
Order : Myrtales
Family : Lythraceae
Genus : *Lawsonia*
Species : *Lawsonia inermis* L.

3.2.14.1 Brief description of the plant

Lowsonia inermis L. or the Henna Plant is native to northern Africa, western and southern Asia, and northern Australia, in the semi-arid zones and tropical areas. It is a glabrous and multi-branched tall shrub or small treewith spine-tipped branchlets. The leaves are opposite; glabrous, sub-sessile, elliptical, and lanceolate, acuminate. Flowers are small, aromatic, white or red coloured with four crumbled petals, numerous; in large pyramidal terminal cyme inflorescence. Fruits are small, brown, in globose capsules[26].

3.2.14.2 Medicinal use of the species

Different parts of the plant are helpful in treating constipating, diarrhoea, dysentery, leprosy, leucoderma, scabies, boils, anemia, hemorrhages, fever, falling of hair, greyness of hair, cough, bronchitis, jaundice and in wound, ulcers, burning sensation, hemicranias, hepatopathy, splenopathy hemoptysisetc[27].

3.2.15 *Syzygium aromaticum* (L.) Merr. & Perry

Source: www.hennasooq.com

Kingdom	**:**	Plantae
Phylum	**:**	Tracheophyta
Class	**:**	Magnoliopsida
Order	**:**	Myrtales
Family	**:**	Myrtaceae
Genus	**:**	*Syzygium*
Species	**:**	*Syzygium aromaticum* (L.) Merr. &L.M. Perry

3.2.15.1 Brief description of the plant

Syzygium aromaticum(L.) Merr.&L.M. Perry or Clove, referred to as Tropical Myrtle, is an evergreen tree that isnative to the MoluccaI slands. The tree grows up to a height of 8–12 m. The branches of the tree are semi-erect with smooth ovatelarge leaves and sanguine flowers grouped in terminal clusters.Theinflorescence is acrogenous thyrses, with about 6mm flower diameter. The flower buds initially have a pale hue, gradually turn green with transition to a bright red hue when ready for harvest. Berries are red-brown, slightly shiny, rectangular oval.

3.2.15.2 Medicinal use of the species

The oil of cloves regulates body temperature and can stimulate the skin by producing heat. Oral administration of this herb can promote the secretion of gastric juice, enhance digestion, relieve nausea and vomiting, reduce abdominal bloating.It also has antiplatelet, anticoagulant, antithrombotic, anti-diarrhoea, choleretic and anti-hypoxia effects[28].

3.2.16 *Achyranthes aspera* L.

Source: www.chhajedgarden.com

Kingdom	**:**	Plantae
Phylum	**:**	Tracheophyta
Class	**:**	Magnoliopsida
Order	**:**	Caryophyllales
Family	**:**	Amaranthaceae
Genus	**:**	*Achyranthes*
Species	**:**	*Achyranthes aspera* L.

3.2.16.1 Brief description of the plant

It is an erect, annual or perennial herb about 1 m to 2 m high which is often found in the moist or shaded areas near trees in savanna or pasture lands. Its stem is angular, ribbed, thickened above the node, more or less glabrous bearing leaves that are opposite, elliptic or obovate, which form an acute or obtuse base with acuminate or rounded apex.Inflorescence is terminal and axillary, spicate, erect, many-flowered; becoming elongate, with only a few flowers opening at the same time. Flowers are hermaphrodite, solitary in axils of acute, membranous, persistent bracts. Fruits are capsuled[29].

3.2.16.2 Medicinal use of the species

A. aspera L. is traditionally used as anti-inflammatory and anti-arthritic agents. It's ingestion helps in asthma, itching, infusion and hysteria. It can be externally used in skin diseases, in foul ulcers, convulsions in children and in the diseases of brain. The roots are also useful in cancer and stones in the bladder[30].

3.2.17 *Hibiscus mutabilis* L.

Source: www.antalyasusbitkileri.com.tr

Kingdom	**:**	Plantae
Phylum	**:**	Tracheophyta
Class	**:**	Magnoliopsida
Order	**:**	Malvales
Family	**:**	Malvaceae
Genus	**:**	*Hibiscus*
Species	**:**	*Hibiscus mutabilis* L.

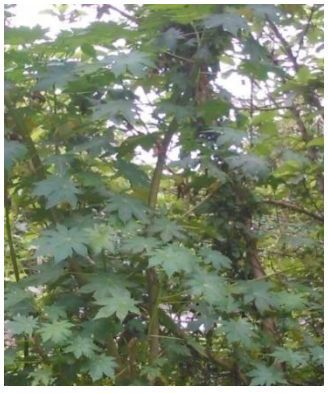
3.2.17.1 Brief description of the plant

Hibiscus mutabili L. is a perennial deciduous shrub that originated from Southeast China. It is a 10-12ft tall shrub with woody stem. The leaves are 3-7 lobed, alternately opposite, pubescent. The flowers are cup-shaped that bloom solitary and change its colour from white in the morning to red gradually as the day progresses[31].

3.2.17.2 Medicinal use of the species

It is very effective in treatment of diabetes. Its flowers are used to cure nasopharyngeal cancer while the leaves are used in tuberculous lymphadenitis. It is also helpful in treatment of swelling and skin infections[32].

3.2.18 *Ricinus communis* L.



Kingdom	**:**	Plantae
Division	**:**	Tracheophyta
Class	**:**	Magnoliopsida
Order	**:**	Malpighiales
Family	**:**	Euphorbiaceae
Genus	**:**	*Ricinus*
Species	**:**	*Ricinus communis* L.

3.2.18.1 Brief description of the plant

Ricinus communis L. or Castor Bean is an evergreen herbaceous or semi-woody large shrub or small tree. The stems are green to reddish-purple in color and have hollow internodes. The leaves are glossy, green to purplish or reddish-green in colour, palmate, with 5-11 deeply incised lobes. Flowers are in inflorescences of terminal racemes or panicles with a few short branches and are glaucous with basally staminate flowers, and apically pistillate flowers per node or a combination of pistillate and (non-opened) staminate flowers. The reddish brown fruits are capsular, falling septicidally apart in 3 bivalved parts[32].

3.2.18.2 Medicinal use of the species

Castor oil and castor seed extractsare used as traditional medicine for disorders like severe constipation, worm infestation, rheumatism, intestinal inflammation. The oil is also used for external application to relieve furuncles, boils, headaches, inflammation of the middle ear and inflammatory skin disorders[33].

3.2.19 *Benincasa hispida* (Thunb.) Cogn.

Source: www.aliexpress.com

Kingdom :		Plantae
Division	**:**	Magnoliophyta
Class	**:**	Magnoliopsida
Order	**:**	Violales
Family	**:**	Cucurbitaceae
Genus	**:**	*Benincasa*
Species	**:**	*Benincasa hispida* (Thunb.) Cogn.

3.2.19.1 Brief description of the plant

Benincasa hispida (Thunb.) Cogn. commonly known as Ash Ground is an extensively trailing or climbimg annual herb growing to a height of 13 to 19 ft. The leaves are large, hispid. The flowers are yellow in colour and solitary. The fruit is cylindrical, glabrous and ash coloured[34].

3.2.19.2 Medicinal use of the species

It is known to be helpful in maintaining sleeping patterns and hence used to treat insomnia. It helps in cystitis and acts as diuretic. It strengthens the lungs and is also used in curing kidney stones [35].

3.2.20 *Solanum nigrum* L.

Kingdom : Plantae
Division : Magnoliophyta
Class : Magnoliopsida
Order : Solanales
Family : Solanaceae
Genus : *Solanum*
Species : *Solanum nigrum* L.

3.2.20.1 Brief description of the plant

This plant is commonly known as Nightshade and is considered to be native to Eurasia. It is an erect, annual or non-rhizomatous perennial shrub that can grow to a height of 1 m. The leaves are elliptical or ovate, entire or slightly lobed throughout, with simple antrorse, curved hairs sparsely distributed on dorsal and ventral surfaces. Flowers are pentamerous in supra-axillary, almost umbellate inflorescence. Fruits are globular in shape[35].

3.2.20.2 Medicinal use of the species

The leaves are traditionally used to cure digestive problems, flatulance, peptic ulcers and dysentery. It is also applied as poultice over rheumatic joints and eruptive skin disorders. A decoction of the berries and flowers is used to treat cough and cold[36].

3.3 Materials and Methods

3.3.1 Reagents

All the media components for cell culture were obtained from Gibco-BRL, Life Technologies Inc., Gaithersburg, MD 20884-9980, USA and Sigma Aldrich, St. Louis, MO, USA. [^{3}H] 2- deoxyglucose (Specific activity: 12.0 Ci/mmol) was purchased from GE Healthcare Bioscience Ltd., Kowloon, HK. Antibodies used *viz.* anti-pAKT1/2/3 and anti-AKT as well as anti-β actin were purchased from Santa Cruz Biotechnology Inc., USA. Alkaline phosphatase conjugated anti-rabbit secondary antibodies were purchased from Santa Cruz Biotechnology Inc., USA. All common chemicals were purchased from Sigma Aldrich, St. Louis, MO, USA, Himedia, Mumbai, India, and BioBharati Life Science, Kolkata, India. Both the analytical and HPLC grade solvents were purchased from Merck, Mumbai, India

3.3.2 Plant materials

The plant materials were collected mostly from the Tezpur University campus, Napaam village of Sonitpur district and various places of Tinsukia and the Dibrugarh district of Assam, India. Based on the traditional knowledge of the local ethnic communities of these places, various parts, e.g. leaves, fruits, roots or barks were collected and washed under tap water until these are clean and then dried in shade. The dried parts were then finely grounded into fine powder using a grinder (Philips, India)

3.3.3 Plant extracts preparation

The ethanolic extracts were prepared for bioavailable active fractionation of the plant parts. Bioactivity of the prepared crude extracts werethen determined. The extraction of the crude extracts was carried out by usingthe cold extraction method with few modifications[37]. Briefly, 300 ml of solvent was added to 60 gms of the powdered sample and kept underconstant stirring for 72 hrs. The solvent was then passed through Whatman filter paper No 1. The filtrate was dried in vacuum using a Rotary vacuum evaporator (Hahn Shin Scientific, Korea). The dried crude extracts were stored in small aliquots at 4°C until use.

Table 3.1: Code used of the different plant extracts

Code used	Plant name	Part used
CECt	*Cinnamomumtamala*(Buch.-Ham.) T. Nees&Eberm.	Leaf
CEMp	*Mimusapudica*L.	Leaf
CEAa	*Achyranthesaspera*L.	Leaf
CELa	*Leucasaspera*(Willd.) Link	Leaf
CENaF	*Nyctanthesarbortritis*L.	Flower
CMHm	*Hibiscus mutabilis* L.	Leaf
CEKp	*Bryophyllumpinnatum*(Lam.) Oken	Leaf
CERc	*Ricinuscommunis*L.	Stem
CECa	*Cassia alata*L.	Leaf
CEPt	*Phlogacanthusthyrsiflorus*Nees	Leaf
CESn	*Solanum nigrum*L.	Fruit
CETc	*Terminaliachebula*Retz.	Fruit
CESa	*Syzygiumaromaticum*(L.)Merr.& Perry	Fruit
CEAsB	*Alstoniascholaris*(L.) R. Br.	Bark
CEAsL	*Alstoniascholaris*(L.) R. Br.	Leaf
CEAc	*Averrhoacarambola*L.	Fruit
CELi	*Lowsoniainermis*L.	Leaf
CESd	*Scopariadulcis*L.	Leaf
CETn	*Trapanatans*L.	Fruit
CENaL	*Nyctanthesarbour-tritis*L.	Leaf
CEPa	*Potentilaanserinae*L.	Leaf
CEBh	*Benincasahispida*(Thunb.) Cogn.	Seed

3.3.4 Cell culture condition and treatments

L6 myotubes were procured from the National Centre for Cell Science (NCCS), Pune, India and cultured in culture flasks containing the DMEM media supplemented with 2mM L-glutamine, 1mM sodium pyruvate, 10% FBS and penicillin (100units/ml), streptomycin (10µg/ml) and incubated at 37^{o}C in a humidified 5% CO_2 environment. The confluent cells were treated without (control) or with 0.75mM palmitate or with bioactive compounds along with palmitate. The palmitate containing media was prepared by conjugation of free

fatty acid with bovine serum albumin (BSA) as described by Dey et al, 2005[38]. Initially, the cells were pretreated with compounds and incubated for 2 hrs followed by 4 hrs of incubation with palmitate. After completion of the incubations, cells were pelleted by centrifugation at 2000 rpm for 5 mins and stored at -20 ºC for further study.

3.3.5 Toxicity study

The MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide) assay was performed to assess the *in vitro* cytotoxicity of the plant extracts or the active compounds using L6 rat skeletal muscle cell line following the method described by Mosmann, 1983[39]. In brief, approximately, $1x10^4$ cells/ml were cultured in complete DMEM media as described earlier. After 80% confluency, the cells were treated with different concentrations of the prepared plant extracts and incubated for 24hrs. After 24 hrs of incubation, MTT solution (5 mg/ml) was added to each well and incubated for 4hrs followed by addition of solvent to dissolve the formed formazone complex. The absorbance was taken at 570 nm and the cell viability was compared to the control cells without any treatment.

3.3.6 Preparation of cell lysate

The cell pellet were resuspended in NP-40 lysis buffer (1% NP-40, 20 mM HEPES (pH 7.4), 2 mM EDTA, 100 mM NaF, 10 mM sodium pyrophosphate, 1mM sodium orthovanadate, 1 µg/ml leupeptin, 1 µg/ml aprotinin, 1 µg/ml pepstatin and 1mM PMSF) and sonicated on ice for 10 mins. Cell lysates were centrifuged at 13,000 rpm for 10 mins at 4ºC. The supernatant was collected and the protein concentrations were determined by the method of Lowry et al, 1951[40].

3.3.7 Electrophoresis and immunoblotting

The proteins present in the cell lysates were subjected to immunoblot analysis. Initially, the lysates containing60 µg of protein were resolved on 10% SDS-PAGE and transferred to PVDF membranes (Millipore) using transfer buffer (pH8.0) for 1.5 hrs. Membranes were blocked with 5% non-fat dried milk in TBST buffer for 2 hrs and incubated with different primary antibodies for 4 hrs. The membrane bound primary antibodies were visualised after incubating with the

secondary antibodies tagged with alkaline phosphatase for 2 hrs followed by developing with the corresponding substrate, 5-bromo 4-chloro 3-indolyl phosphate/nitro blue tetrazolium (BCIP/NBT). Image of the bands were observed using gel documentation apparatus (BioRad, USA). Band intensities were quantified by available GelQuant.NET v 1.6.8software.

3.3.8 [^{3}H] 2- Deoxy Glucose Uptake Assay

The [^{3}H] 2- Deoxyglucose (Amersham Biosciences, USA) uptake in L6 myotubes was conducted as described by Barma et al, 2006 [41]. Briefly, L6 myotubes were serum starved overnight in Kreb's Ringer Phosphate (KRP) buffer, pH 7.4 supplemented with 0.2% BSA. Cells were incubated for 6 hrs with or without 0.75 mM palmitate in presence of the plant extracts followed by 30 mins incubation with porcine insulin (100 mM). Incubations without any of these chemicals were taken as the control. [^{3}H] 2- deoxyglucose (0.4 mmoles/ml) was added to each condition and allowed to incubate for 5 mins before the termination of the incubation. Uptake of the glucose was stopped by washing the cells thrice with ice cold KRP buffer (pH) in presence of 0.3 mM phloretin to correct the glucose uptake data. Cells were harvested with trypsin (0.25%)- EDTA (0.5 mM), solubilised with 1%NP-40 and [^{3}H] 2- deoxyglucose (2-DOG) was measured in a Liquid Scintillation counter (Perkin Elmer, Tricarb 2800 TR).

3.3.9 Measurement of total polyphenolic contents in crude ethanolic extract of *L. aspera* (Willd.) Link (CELa)

The total polyphenolic (TPC) in the crude ethanolic extract of *L. aspera* (CELa) was determined by the modified Folin-Ciocalteu method[42]. Briefly, in 50 µl of the extract, 5 ml of Folin-Ciocalteu reagent (1:10v/v) and 4 ml (75 mg ml^{-1}) of sodium carbonate solution was added and allowed to incubate for 30 min at 40°C. Absorbance of the reaction mixture was measured in a UV-Vis spectrophotometer (MultiSkan Go, Thermoscientific) at 765 nm. The total phenolic content was expressed as milligram of gallic acid equivalent/mg (mg GAE/mg). The gallic acid standard curve was established by plotting concentration (mg ml^{-1}) versus absorbance (765 nm) calculated as, y= 0.0266+0.037, R^2=0.9821 where y is the absorbance and x is the gallic acid equivalent.

3.3.10 Estimation of total flavonoid content (TFC) in crude ethanolic extract of *L. aspera* (Willd.) Link (CELa)

The total flavonoid content (TFC) of CELa was determined following the method of Shekhar et al.[43] Briefly, 0.5 ml of 2% $AlCl_3$ (w/v) ethanolic solution was mixed with 0.5 ml of the extract. The reaction mixture was incubated for 1 hr at room temperature and the absorbance was measured at 420 nm in a UV-VIS spectrophotometer (MultiSkan Go, Thermoscientific). TFC was calculated as milligram of quercetin equivalent/mg (mg QE/mg) using the following equation based on the calibration curve, y=0.0866x +0.1124, R^2= 0.9678, where y is the absorbance and x is the quercetin equivalent.

3.3.11 Bioassay guided fractionation

3.3.11.1 General Consideration

In natural products drug discovery program bioassays are applied to large numbers of initial samples for preliminary screening to determine whether or not the samples have any bioactivity of the desired type. Also, it helps in guide fractionation of a crude material towards isolation of the pure bioactive compounds. Hence, for these purposes, bioassay tests must be simple, rapid, reliable, reproducible, sensitive, most importantly, predictive.

3.3.11.2 Column Chromatography

The crude ethanolic extract of *L. aspera* was subjected to column chromatography for fractionation. Silica gel of mesh 100-200was used with petroleum ether to pack the column using a pasteur pipette and allowed to stand for 6 hrs.Then the extract was added to the top of the column. Four different solvents of different polarity, *viz.* petroleum ether, chloroform, ethanol and water were used as eluents. Four different fractions were collected separately and evaporated in a rotary evaporator (Hahn Shin, Korea) followed by bioactivity.

3.3.11.3High performance liquid chromatography (HPLC)

The active fractions were further purified using High Pressure Liquid Chromatography (HPLC) using a Waters HPLC system with a binary 515 Pump system and a 2489 UV-VIS detector. A 250mm semi-preparative reverse phase

HPLC column (Symmetry C_{18}) (4.6 x 250 mm) was used for the separation and purification of the compounds. Water (pump A) and methanol (pump B) were used as the solvent system to separate the peaks using the gradient method given in the Table 3.2. The flow rate was maintained at 1 ml/min and the detection was carried out at wavelength 350 nm.

Table 3.2: HPLC method used for separation of the peaks

Time (Min)	% of A (Water)	% of B (Methanol)
0	100	0
10	100	0
15	70	30
55	0	100
65	0	100
70	100	0
80	100	0

3.3.11.4UV Spectroscopy

A Thermo Scientific UV-Vis spectrophotometer was used to determine the absorption maxima (λ max) of the compounds. The λ max gives a brief idea on the presence of different chromophores (molecules in compounds that absorb light) in the compound. Methanol was taken as the blank and the compounds were scanned from 200-800 nm to determine the λ max.

3.3.11.5LC-MS analysis

For LC-MS analysis, Agilent 1260 Infinity HPLC-Chip/MS System was used. Water and acetonitrile were used as the solvent system to run with a flow rate of 0.30 µl/min. An amount of 3 µl of the sample was allowed to inject for run the scan. The scan rate was set 1 spectra/min and a mass range of 60-1000 (m/z) was scanned.

3.3.11.6Fourier Transformer Infra Red Spectroscopy (FTIR)

The FT-IR spectroscopy analysis was performed to determine the differentfunctional groups present in the compounds, which enabled partial identification of the compounds. The compounds were first mixed with about 5-7 mg of potassium bromide (KBr) to obtain a fine mixture. This was then taken in a sample holder and then placed under a hydraulic press. The hydraulic press delivers 10 ton pressure to produce a KBr compound tablet which was then placed in the sample holder for subsequent characterization. The FT-IR transmittance spectra were obtained using a Perkin Elmer Spectrum 100 series instrument.

3.3.11.7Nuclear Magnetic Resonance (NMR)

Both ^{1}H NMR and ^{13}C NMR were carried out using JEOL FT 400 MHz NMR. Compounds were dissolved in NMR grade methanol for the study. Chemical shifts were presented as δ (ppm) and coupling constants (J) as Hz.

3.4 Results

3.4.1 Standardization of dose of saturated fatty acid and time of incubation

To study the antidiabetic activity of the extracts and the compounds against FFA induced insulin resistant model, palmitate was used as inducer. L6 skeletal muscle cells were treated with 3 different concentrations of palmitate for 24 hrs followed by 30 mins incubation with insulin. The cells were harvested and the lysates of the harvested cells were subjected for immunoblot analysis using anti pAKT and anti AKT antibody. It was observed that palmitate reduced the insulin stimulated AKT phosphorylation with increasing concentration of palmitate. Densitometric analysis of two independent experiments revealed that 0.75 mM palmitate can reduce approximately two fold of insulin stimulated AKT phosphorylation than insulin treated L6 skeletal muscle cells (Fig. 3.1 A).

Palmitate incubation time was optimized by treatment of the L6 skeletal muscle cells with 0.75 mM palmitate for 3 different time periods. Immunoblot analysis of the cell lysates revealed that 4 hrs of incubation with 0.75 mM palmitate can significantly reduce the insulin stimulated AKT phosphrylation approximately two fold than the insulin treated L6 cells (Fig. 3.1 B)

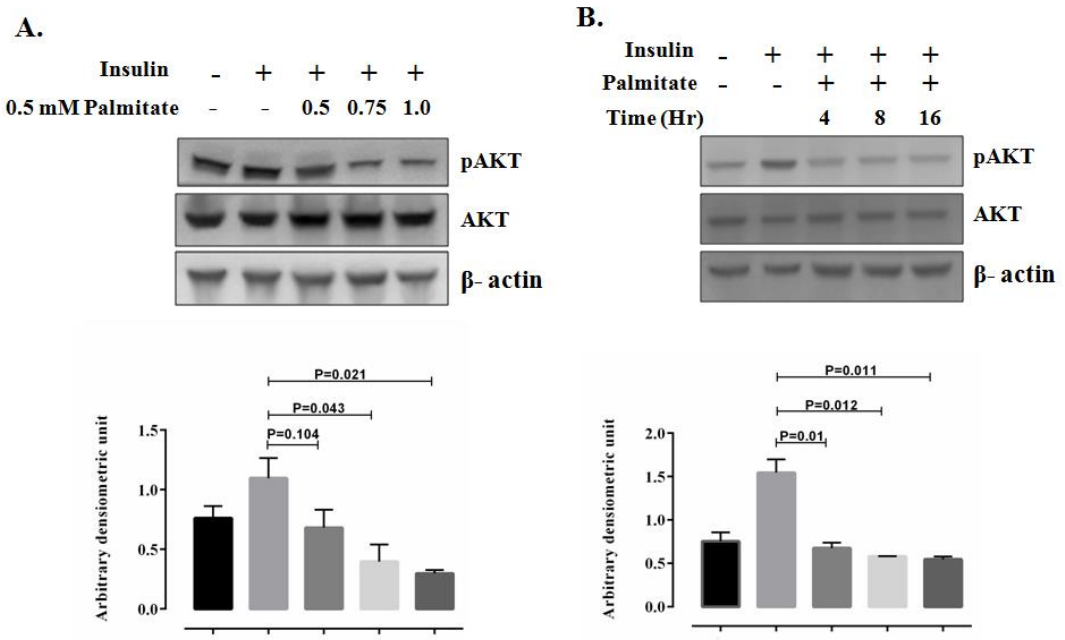


Figure 3.1: Standardization of the dose of palmitate and time of incubation.(A) L6 myotubes were treated for 24 hrs with various concentrations of palmitate or without plamitate followed by 30 mins incubation with insulin and (B) L6 myotubes were treated for various time periods with 0.75 mM concentrations of palmitate or without plamitate followed by 30 mins incubation with insulin. Immunoblot analysis was performed using anti-pAKT and anti-AKT antibody. Beta-actin was used as loading control. Densiometric analysis carried out by using Gel quant software. Each value is the Mean ± SEM of two individual experiments and the p value were calculated using GraphPad PRISM v 6.07

3.4.2 Cytotoxicity study of the plant extracts

Twenty plants were considered for initial screening on the basis of their activity against FFA induced insulin resistance. At the beginning, the cytotoxic effect of all the extracts was studied where the L6 myotubes were treated with 400 µg/ml concentration of the extracts independently for 24hrs. After 24 hrs of treatment the cells were exposed to MTT solution for 4 hrs. The cell viability varied remarkably with various extracts. But atleast 60% of viability was found in all the cells independent of the extracts (Fig. 3.2). So, all the extracts were considered for further experiment to check their effect on insulin stimulated AKT phosphorylation in presence of FFA.

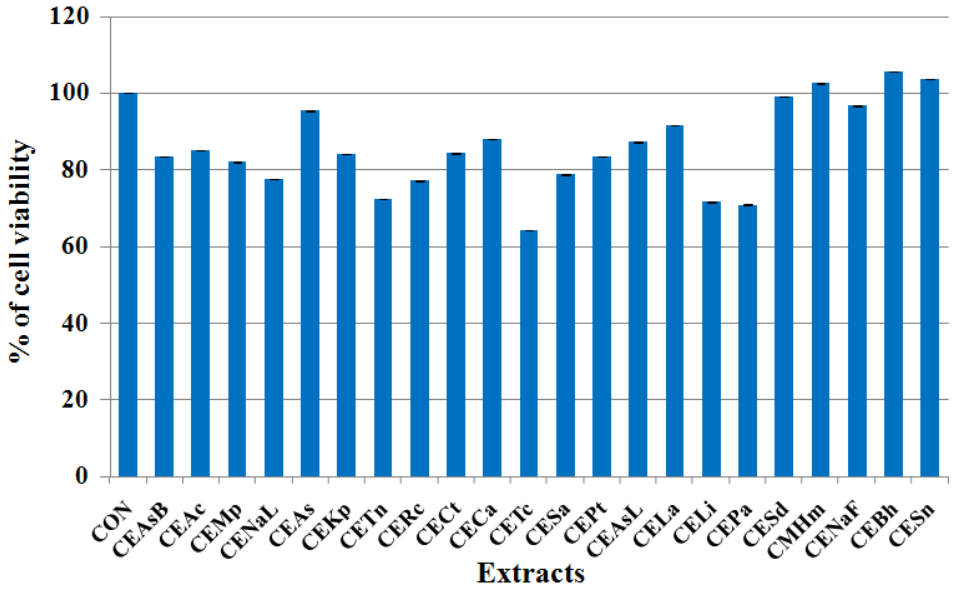


Figure 3.2: Percentage of cell viability after the treatment of various plant extracts. L6 cells were treated with 400 µg/ml of different plant extracts for 24 hrs and viability was observed followed by the MTT method. Cells without any treatment were used as control.

3.4.3 Screening of the traditionally used antidiabetic plants having insulin sensitive property

L6 myotubes were treated with palmitate and various plant extracts in the presence and absence of insulin and incubated for 4 hrs. After incubation the cells were harvested and the cell lysates were subjected to western blotting. The effect of the extracts on the phosphorylation of AKT protein was studied.β actin was used as the loading control. AKT phosphorylation was considered because it is one of the major intermediators in the insulin signalling pathway. It is well documented that palmitate reduces the phosphorylation of AKT which leads to the reduction in GLUT4 translocation to the cell membrane in presence of insulin. From the western blot analysis, it was observed that the extracts CEAa, CELa, CEHm, CEPt, CERc, CECa, CELi, CESd, CETn, CENaL, CEPa, CEBh, CETc, CESa, CEAsB, CEAsL and CEAccould restore AKT phosphorylation in presence of FFA and insulin, while the remaining extracts showed negligible activity. Densitometric analysis was performed and based on the result, ethanolic extracts of *Leucas aspera* (Willd.) Link (CELa) and *Phlogacanthus thyrsiflorus* Nees. (CEPt) were found to be more effective (Fig 3.3).

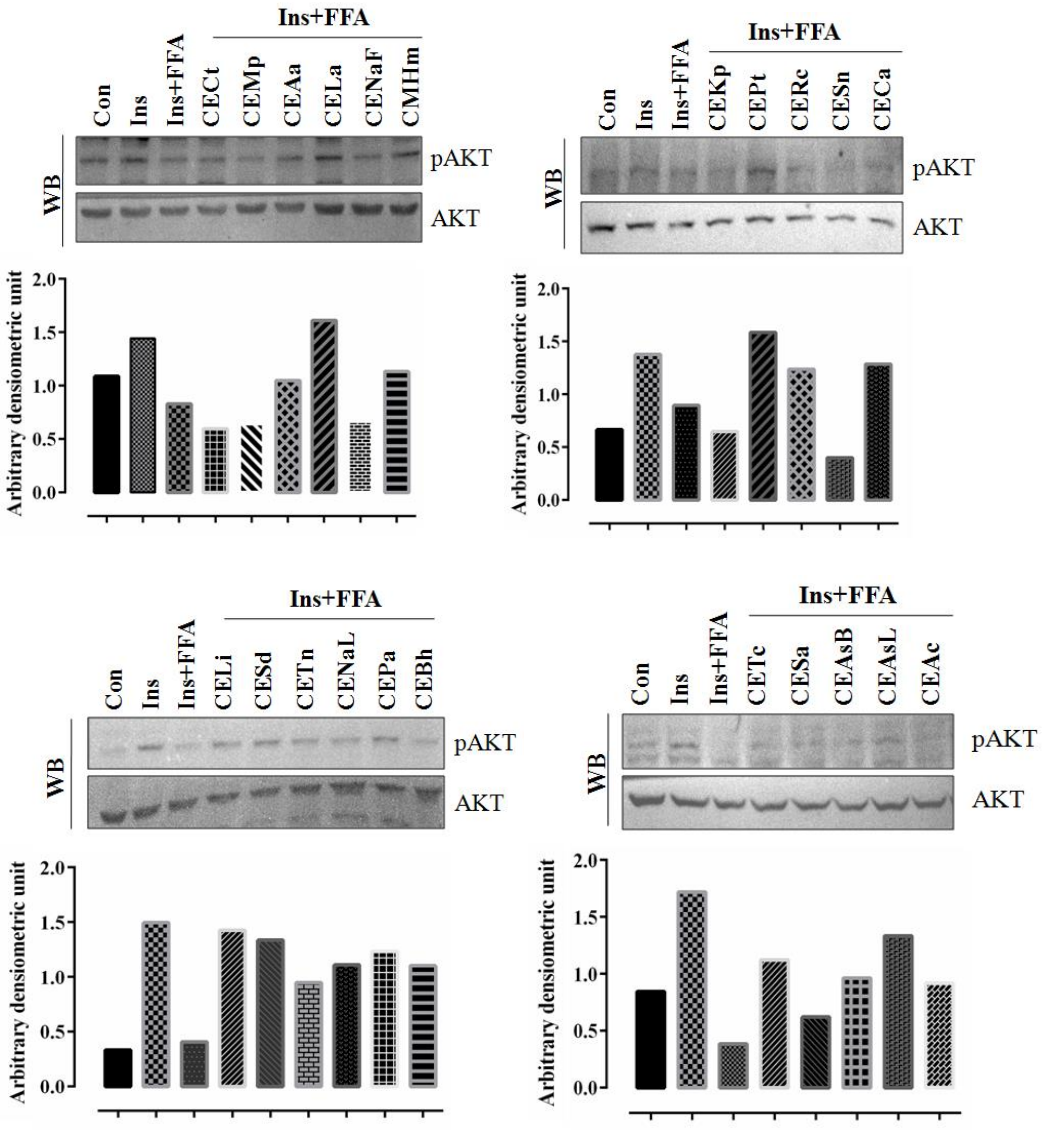


Figure 3.3: Western blot analysis showing AKT phosphorylation by different plant extracts. L6 muscle cells were pretreated with different plant extracts (200 µg/ml each) for 2 hrs followed by 4 hrs incubation with 0.75 mM FFA. The cell lysates were subjected to western blot analysis using anti-pAKTand anti-AKT antibodies. Densitometric analysis was carried out by using GelQuant.NET software v 1.6.8.

3.4.4 Improvment of glucose uptake activity of the cells by the treatment with the crude ethanolic extract of *L. aspera* (CELa) and crude ethanolic extract of *P. Thyrsiflorus* (CEPt)

Based on the previous result, where the crude ethanolic extracts of *L. aspera* (CELa) and *P. Thyrsiflorus* (CEPt) showed highest activity to improve the prosphorylation of AKT in presence of palmitate, it was hypothesized that these extracts may have insulin stimulated glucose uptake activity.To study this, ^{3}H-

2deoxy glucose (2DOG) uptake assay was performed using CELa and CEPt in presence of palmitate. Form the 2DOG uptake assay it was observed that both the extract have the ability to improve palmitate induced impairment of glucose uptake. CELa was found more potent than the other (Fig 3.4). Based on this result CELa was selected for further studies.

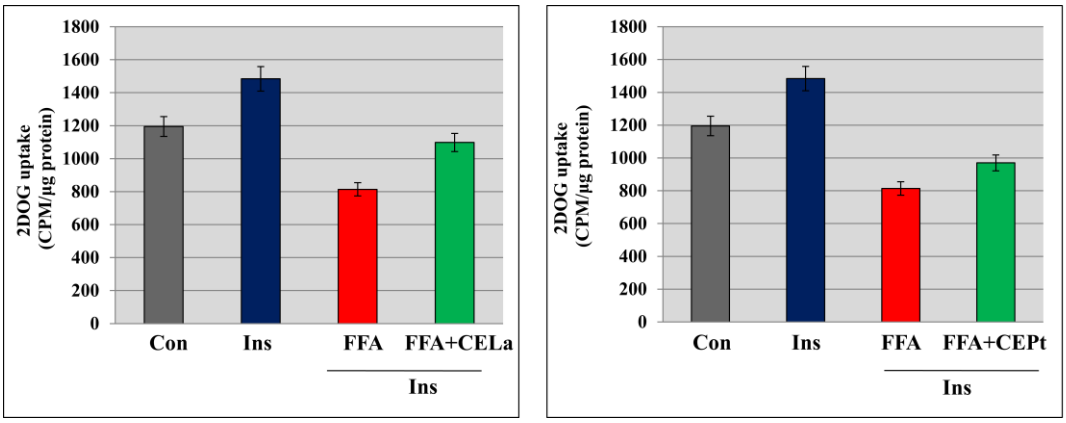


Figure 3.4:^{3}H- 2deoxy glucose (2DOG) uptake assay was performed using CELa and CEPt. L6 myotubes were treated for 4 hrs with plamitate or palmitate along with *L. aspera* ethanolic extract (CELa) and *P. thyrsiflorus* ethanolic extract (CEPt) or without any of them (control) followed by 30 mins incubation with insulin. ^{3}H-2deoxyglucose was added 5 mins prior to the termination of incubation and the radioactivity was counted in liquid scintillation counter.

3.4.5 Phytochemical analysis of the crude ethanolic extract of *L. aspera* (CELa)

The quantitative determination of the total phenolic (TPC) and total flavonoid (TFC) content in the crude ethanolic extract of *L. aspera* (CELa) was carried out to correlate it with antioxidative properties. The analysis showed that the TPC in the CELa extract was found to be 0.072 mg gallic acid equivalent/mg (GAE/mg) dry weight basis (DWB) and the amount of flavanoid content was found to be 0.22 mg quercetin equivalent/mg (QE/mg) DWB.

3.4.6 Isolation of bioactive compounds from the crude ethanolic extract of *L. aspera* (CELa)

3.4.6.1 Column chromatography

The crude ethanolic extract of *L. aspera* (60 gms) was subjected to column chromatography using silica gel (100-200 mesh) with petroleum ether, chloroform, ethanol and water to obtain 3 fractions and named as LAPE, LAC and LAE

respectively. The fractions eluted with ethanol, i.e., LAE showed the best bioactivity as it could restore AKT phosphorylation in presence of 0.75 mM palmitate as well as insulin (Fig. 3.5). This fraction was considered for further purification as AKT activation modulates the initiation of GLUT4 translocation to the cell membrane.

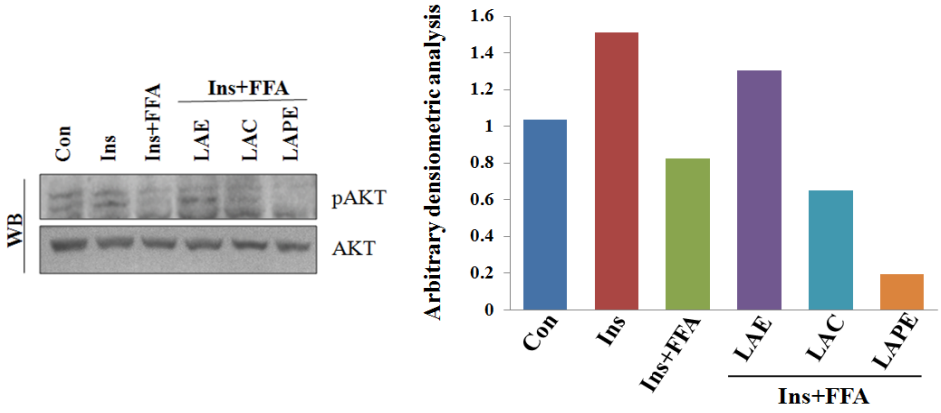


Figure 3.5: Western blot analysis showing AKT phosphorylation by different fractions of CELa.L6 muscle cells were pretreated with different fractions of CELa (50 µg/ml each) for 2 hrs followed by 4 hrs incubation with 0.75 mM FFA. The cell lysates of each incubation were subjected for SDS-PAGE followed by westerm blot analysis using anti-pAKTand anti-AKT antibodies. Densitometric analysis was carried out by using GelQuant.NET software v 1.6.8.

3.4.6.2 High Performance Liquid Chromatography (HPLC)

Reverese phase HPLC (RP-HPLC) method coupled to a UV-Vis detector was used simultaneously to detect the different components present in the active LAE fractions of *L. aspera*. A semi-preparative reverse phase HPLC column of dimension 4.6 x 250 mm (Symmetry C18) (5µm) was used for the separation and purification of the compounds present in the LAE fraction of CELa. Fractionation of the extract was carried out using a gradient of 0-100% methanol in 80 mins keeping the flow rateat 1 ml/min. The elution was monitored at 350 nm. A total of 16 fractions were obtained from the LAE and all the fractions were collected separately and named as Ela 1-16 (Fig. 3.6). All the fractions were checked for bioactivity by immunoblot analysis using the anti-pAKT antibody (Fig. 3.7) and the insulin stimulated 2-deoxyglucose (2-DG) uptake assay (Fig. 3.8).

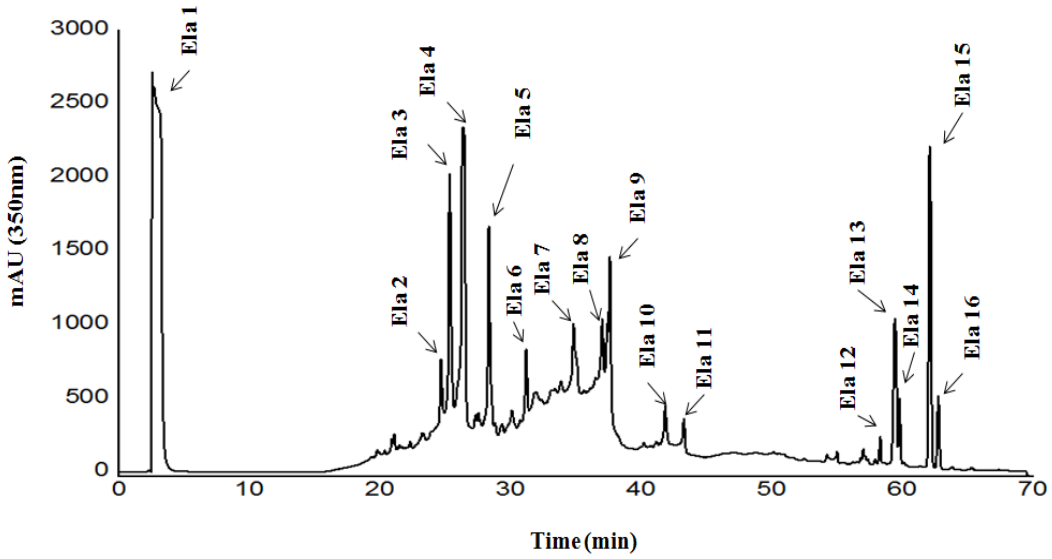


Figure 3.6: HPLC Fractionation of ELA. ELA was loaded into a C18 column (4.6 x 250 mm, particle size, 5 µm pore size). The column was equillibrated with milliQ water and fractionation was carried out using a gradient of 0- 100% methanol in 80 mins. Flow rate was 1ml/min and elution was monitored at 350 nm.

3.4.6.3 Effect of HPLC purified fractions on FFA induced inhibition of AKT phosphorylation and insulin stimulated 2-deoxyglucose (2-DG) uptake

All the 16 fractionsobtained from the HPLC were then subjected to bioactivity analysis in insulin stimulated AKT phosphorylation in presence of 0.75 mM FFA. The immunoblot analysis revealed that a total of 7 HPLC purified fractions*viz.*Ela3, Ela4, Ela5, Ela7, Ela10, Ela14 and Ela 15 have some modulatory activity in restoring AKT phosphorylaion in presence of FFA (Fig 3.7)

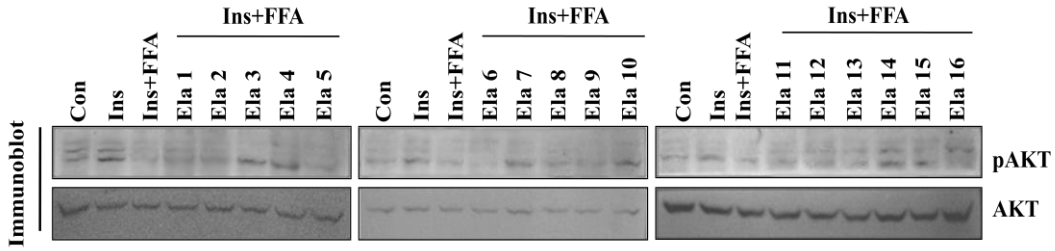


Figure 3.7: Western blot analysis showing AKT phosphorylation by the different HPLC purified fractions of the LAE. L6 muscle cells were pretreated with different fractions of the LAE (20 µg/ml each) for 2 hrs followed by 4 hrs incubation with 0.75 mM FFA. The cell lysates of each incubations were subjected for SDS-PAGE followed by westerm blot analysis using anti-pAKT antibody where anti-AKT antibody was used as loading control.

Since the glucose uptake by the cells is one of the major functions of insulin to maintain the glucose homeostasis in the body, the glucose uptake in FFA treated L6 muscle cells were checked by the action of the 7 HPLC purified active fractions using Glucose Uptake Colorimetric Assay kit. From the result it was observed that, Ela7showed the best activity of insulin stimulated glucose uptake in presence of palmitate in L6 skeletal muscle cells (Fig. 3.8)

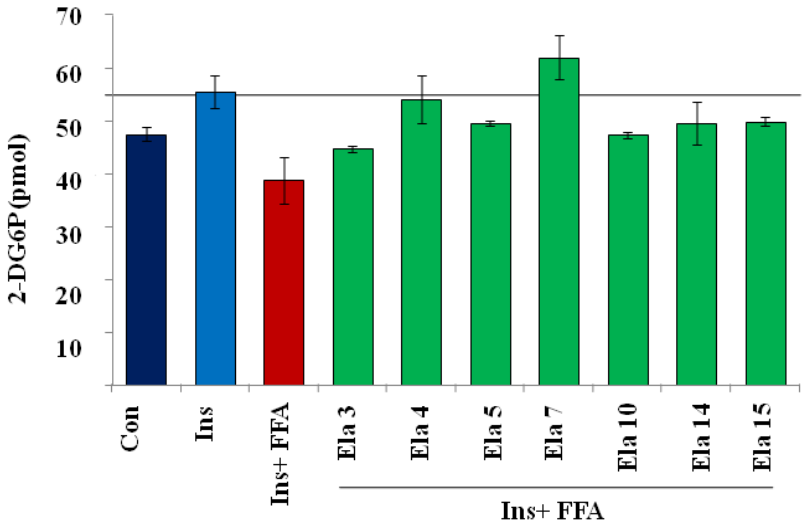


Figure 3.8: Insulin stimulated 2-deoxyglucose (2-DG) uptake assay. L6 myotubes were treated for 4 hrs with plamitate or palmitate along with 7 HPLC purified active fractions having modulatory effect on insulin stimulated AKT phosphorylation (20 µg/ml each) or without any of them (control) followed by 30 mins incubation with insulin. 2-DG (10 mM) was added 20 mins prior to the termination of incubation following the manufacturer protocol and measured the absorbance at 420 nm at microplate reader.

3.4.6.4 Characterization of bioactive compounds

The absorption maxima (λmax) of the fraction Ela7 was analyzed with the help of a UV-Vis Spectrophotometer (Thermo-Fischer). Thecompound was dissolved in methanol and scanned from 200-800 nm to obtain the λmax. The λmax of the compound was found to be 330 nm (Fig. 3.9)

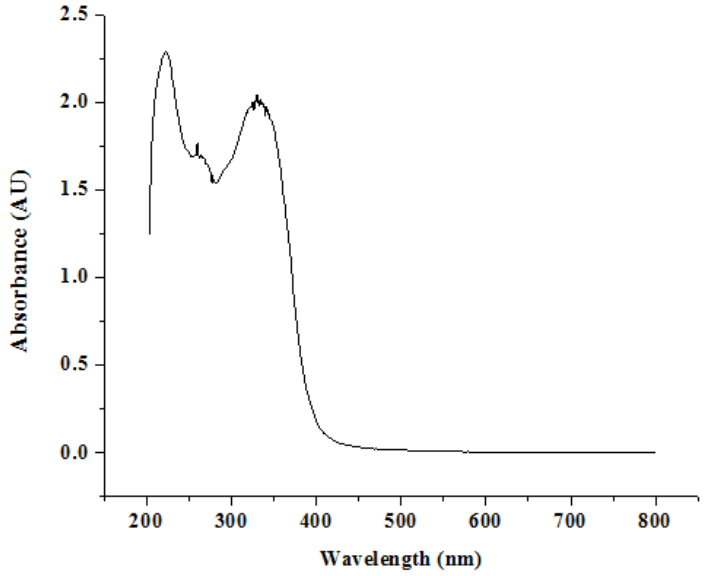


Figure 3.9: λmax spectra of the purified Ela7

3.4.6.5 High Resolution Liquid Chromatography Mass Spectroscopy (HR-LCMS)

HR-LCMS analysis suggested a group of compounds which can be of Ela7. The probable compounds suggested by HR-LCMS are listed in the Table 3.3. The 2D structures of all the compounds were analysed with the characteristic peaks of Ela7 obtained from FTIR and NMR (both ^{1}H and ^{13}C).

Table 3.3: List of compounds suggested by HR-LCMS analysis

Compound Name	Mass
Bis (2-hydroxypropyl) amine	162.11
Ethyl Oxalacetate	188.06
Sulfolithocholylglycine	513.27
Dihydrorobinetin	304.05
Petunidin	317.06
10-Deoxymethymycin	453.30
(24S)-24-fluoro-1alpha,25dihydroxyvitamin D2 / (24S)24-fluoro-1alpha,25dihydroxyergocalcifero	446.32
Acetylaminodantrolene	326.09
Lecanoric acid	318.07
8Z,11Z,14Zheptadecatrienoic acid	264.20
2,5-octadecadiynoic acid	276.20
PGF2alpha-1,11-lactone	336.23
Swietenine	568.26
Dihydrodeoxystreptomycin	567.28
Pristimerin	464.30
Clovanedioldiacetate	322.21
Lactone of PGF-MUM	296.15
GPGro(16:0/0:0)[U]	484.27
27-nor-5b-cholestane3a,7a,12a,24,25-pento	438.33
1alpha-hydroxy-24(dimethylphosphoryl)25,26,27-trinorvitamin D3 /1alpha-hydroxy-24(dimethylphosphate)	434.29
3beta,6alpha,7alphaTrihydroxy-5beta-cholan-24oic acid	408.28

3.4.6.6 Fourier Transform Infra Red Spectroscopy (FTIR)

The FTIR spectroscopy for Ela7 was carried out toidentify the functional groups present in the fractions. The compound exhibited multiple transmittance peakssuggesting the presence of different functional groups. FTIR analysis showed the characteristic transmittance peaks at wavenumber 3443, 2919, 2850, 1631, 1461, 1386 and 1050 cm^{-1}(Fig. 3.10). These peaks indicates presence of –OH stretching, −CH symmetric and asymmetric stretching of $-CH_2$ groups, C=O stretching, −CH2 bending and wagging vibrations and C−O−C stretching vibration of ester groupin the compound. However, the FTIR data complements other methods for structure determination of the compound

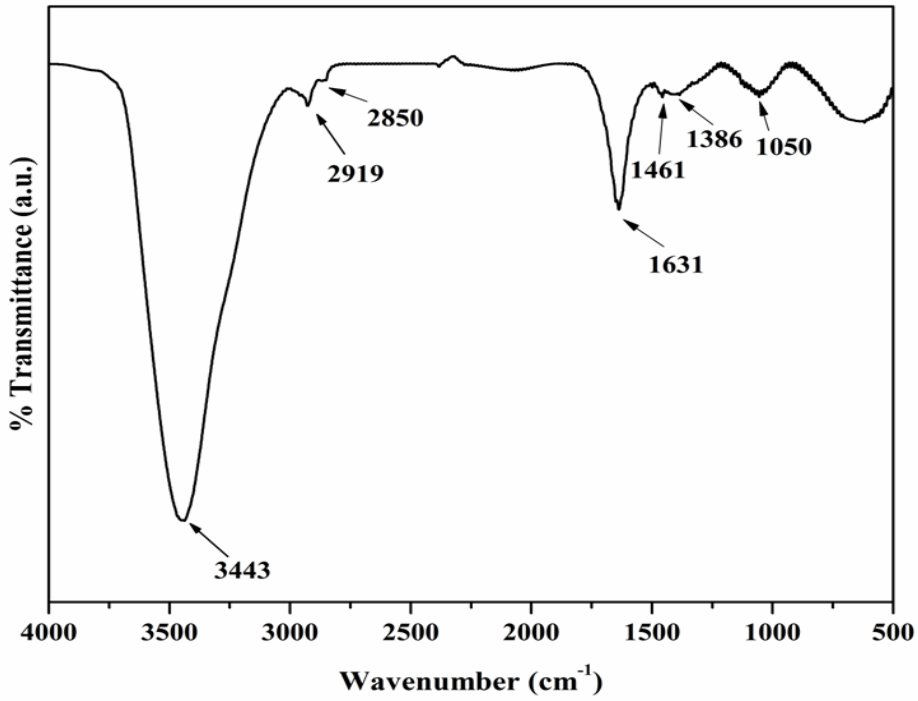


Figure 3.10: FTIR spectra of Ela7

3.4.6.7 Nuclear Magnetic Resonance (NMR) analysis

NMR spectroscopy is an important tool of choice in structure elucidation ofunknown compounds. Both ^{1}H and ^{13}C NMR experiments were carried out. A total of 8 scans were given for ^{1}H NMR and 20,000 scanswere given for ^{13}C NMR to run of the samples. The spectral peaks of both the ^{1}H and ^{13}C NMR are shown in the Fig. 3.11 and the peaks that obtained from both the NMR are listed in the Table 3.4

Table 3.4: ^{1}H NMR and ^{13}C NMR spectral data of Ela7

^{1}H NMR (δ H) (ppm)	**^{13}CNMR (δ C) (ppm)**
0.87	13.13
1.23	29.12
1.26	29.15
1.28	29.30
1.35	29.37
1.41	29.45
2.12	31.75
2.22	47.46
2.24	47.67
3.28	47.89
4.85	124
6.52	126
6.64	128
	130
	176.70
	208.86

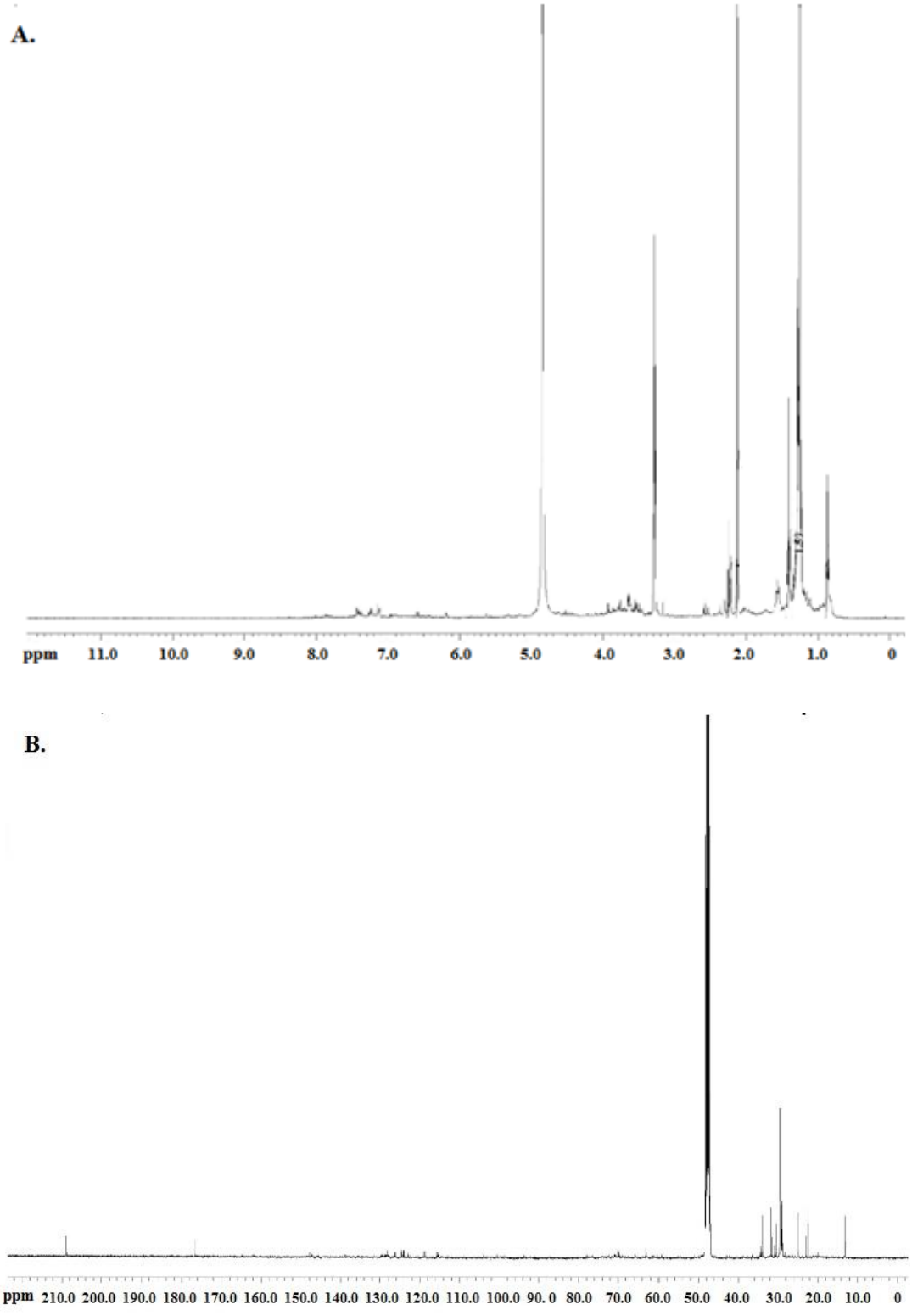


Figure 3.11: NMR spectra of Ela7. (A) ^{1}H NMR spectra of Ela7 and (B)^{13}C NMR spectra of Ela7.

3.4.6.8 Probable structure of the bioactive compound

Based on the results of the UV-Visible spectra, FTIR and NMR (^{1}H NMR and ^{13}C NMR) and HR-LCMS, the active component present in Ela7 was identified. The characteristic peaks formed due to the presence of different functional groups and spectral pattern of H and C present in the compound were analysed with the structure of all the compounds suggested by HR LCMS analysis. After the structural analysis and based on the previous literatureof Dewanjee et al., 2009,the compound is predicted as swietenine[44]. The 2D structure of swietenine is shown in the fig. 3.12.

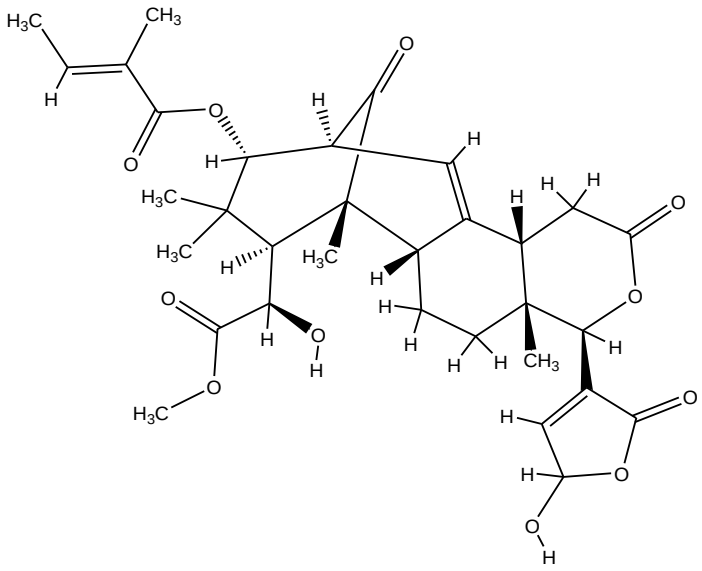


Figure 3.12: 2D structure of Swietenine

3.5 Discussion

Attempts have been made in this investigation to obtain the active antidiabetic compound(s) from plant based sources depending upon the traditional knowledge.The dose of palmitate (as FFA) and the time of incubation was optimized to make the cells insulin resistant based on the significant reduction of insulin stimulated AKT phosphorylation. AKT phosphorylation is the key event for GLUT4 translocation to the membrane. Twenty different traditionally used antidiabetic plants were screened by investigating their ability to induce insulin stimulated AKT phosphorylation in presence of FFA. Of all the extracts studied, *P. thyrsiflorus* and *L. aspera* showed the most promising effect. Restoration of AKT phosphorylation at Thr 308 by extracts of *P. thyrsiflorus* and *L. aspera* were found

to be more than the insulin treated cells. Thr308 is the main catalytic domain of AKT and is activated by PDK1. The activation of AKT kinase leads to its dissociation from the plasma membrane and activates many substrates which are directly or indirectly involved in insulin dependent pathways[45]. Between these two plants, *L. aspera* showed better activity in insulin stimulated [^{3}H]-2' deoxy glucose uptake in cells treated with FFA. So, further fractionation and isolation of the bioactive compound was carried out using crude ethanolic extract of *L. aspera* (CELa). Initially, the CELa was subjected for column chromatography using 3 different solvents on the basis of their polarity followed by immunoblot analysis to check their activity in insulin induced phosphorylation of AKT in presence of FFA. The active ethanolic fraction of CELa showed the best activity and hence further purification was carried out with the active ethanolic fraction of CELa (LAE). The LAE was then subjected for HPLC purification using RP-C18 column. A total of 16 peaks were observes and collected separately. All the peaks were tested for the bioactivity in insulin stimulated AKT phosphorylation in presence of FFA by immunoblot analysis. The 7 fractions showing the restoration of insulin stimulated AKT phosphorylation in FFA treated cells were further investigated for insulin stimulated glucose uptake in FFA treated L6 skeletal muscle cells and fraction 7 of HPLC (Ela7) was found to be the most active fraction for insulin stimulated glucose uptake. Ela7 was further characterised by HR-LCMS, FTIR and NMR analysis. HR-LCMS analysis of Ela7 suggested several probable compounds and each compounds were critically analysed with the peaks of FTIR and NMR (both ^{1}H NMR and ^{13}C NMR).

In the FTIR spectrum, a broad peak cantered at 3443 cm^{-1} can be assigned –OH stretching. Absorption peaks near at 2919 cm^{-1} and 2850 cm^{-1} are assigned to the −CH symmetric and asymmetric stretching vibrations of $-CH_2$ groups, respectively. An intense peak at 1631 cm^{-1} is due to the C=O stretching vibration. The peaks at 1461 and 1386 are attributed to −CH2 bending and wagging vibrations, respectively. The peak associated with C−O−C stretching vibration of ester group is observed at 1050 cm^{-1}.

In the^{1}H NMR spectrum, the peak at δ 0.87 ppm is assigned to the protons of the terminal methyl group. Protons of $-CH_2$ groups are observed at δ 1.23–1.28

ppm and that of –CH group are appeared at δ 1.35–1.42 ppm. The peak for the –OH proton appeared at δ 2.12–2.24 ppm. The peaks at δ 3.28–3.84 ppm corresponding to the protons of $–CH_2$ and $–CH_3$ moiety bonded with an electron withdrawing oxygen atom. The peak at δ 4.85 ppm can be assigned to the protons of –CH moiety bonded with and C=O group and oxygen atom. The protons of the C=C moiety is observed at δ 6.52–6.64 ppm.

In ^{13}C NMR spectrum chemical shift at δ 208 and 176 ppm are corresponding to carbonyl group (C=O) of ketone and ester, respectively. The peaks in the range of δ 120−130 ppm are corresponding to the carbon atoms of unsaturation moieties. The peaks in the range of δ 13−47 ppm assigned to the saturated carbon atoms.

From the spectral data obtained from FTIR, ^{1}H NMR and ^{13}C NMR and analysing the peaks pattern and properties it can be concluded that the active compound is swietenine. The hypoglycaemic effects of swietenine have been reported earlier. So, from literature review and our data, it can be strongly suggested that the probable active compound may be swietenine which induced insulin stimulated AKT phosphorylation as well as glucose uptake in presence of FFA in L6 skeletal muscle cells.

References:

1. Mahabir, D. & Martin, C. Gulliford, Use of medicinal plants for diabetes in Trinidad and Tobago, *RevistaPanamericana de SaludPública***1**(3), 174--179, 1997.
2. Grover, J. K. & Vats. V., Shifting paradigm: from conventional to alternative medicines-an introduction on traditional Indian medicine, *Asia-Pacific Biotech News* **5**(1), 28--32, 2001.
3. WHO, 56 World Health Assembly, http://www.who.int/mediacentre/news/notes/2003/npwha/en/, 2003.
4. Hummel, M. & Kiviat, E., Review of world literature on water chestnut with implications for management in North America,*Journal of Aquatic Plant Management*, **42,** 17--28, 2004.
5. Mehrhoff, L.J., etal.*Trapanatans. IPANE: Invasive plant atlas of New England*,Storrs, CT, University of Connecticu, 2003.
6. Ghani, A., et al. Physico-chemical, Morphological and pasting properties of starches extracted from water chestnuts (Trapanatans) from three lakes of Kashmir, India, *Brazilian Archives of Biology and Technology***53**(3), 731--740, 2010.
7. Rahman, M. M., et al. Antimicrobial activity and cytotoxicity ofTrapabispinosa, *Fitoterapia***71**(6), 704--706, 2000.
8. Fundter, J.M., et al. Terminaliachebula Retz., in*Plant Resources of South-East Asia No 3. Dye and tannin producing plants.Pudoc*, Lemmens, R.H.M.J.,&Wulijarni-Soetjipto, N., eds., Wageningen, Netherlands,1991, 122–125.
9. Bag, A., et al. The development of Terminaliachebula Retz. (Combretaceae) in clinical research, *Asian Pac J Trop Biomed***3**(3), 244--252, 2013.
10. Orwa, C., et al. *Agro-forestreeDatabase:a tree reference and selection guide version 4.0*, 2009
11. Kaushik, P., et al. *Alstoniascholaris*: It's Phytochemistry and pharmacology, *Chronicles of young scientist***2**, 71--78, 2011

12. Khyade, M. S., et al. Alstoniascholaris (L.) R. Br. and Alstoniamacrophylla Wall. ex G. Don: A comparative review on traditional uses, phytochemistry and pharmacology, *Journal of ethnopharmacology* 153(1),1--18, 2014.
13. Chin, W.Y. *A Guide to Medicinal Plants*, BP Singapore Science Centre, 1992, 33
14. Champa, R., et al. Nyctanthesarbor-tristis Linn (Night Jasmine): A sacred ornamental plant with immense medicinal properties, *Indian Journal of Traditional Knowledge*, **11**(3), 427--435, 2012.
15. Saxena, R.S., etal.Tranquilizing, antihistaminic and purgative activity of *Nyctanthesarbortristis* leaf extract, *J Ethnopharmacol* **81**(3), 321--325, 2002.
16. Phurailatpam,A.K.,et al. *Phlogacanthus*- An important medicinal plant of North East India: A review, *African journal of Agricultural Research* **9**(26), 2068--72, 2014.
17. Taylor & Gladys. *Saints and their Flowers*, A. R. Mowbray & Co., Oxford,London, England, 1956.
18. Dharmananda, S., *Silverweed: Potentilla species used in herbal medicine*, Institute for Traditional Medicine, Portland, Oregon, http://www.itmonline.org/arts/silverweed.htm
19. Prajapati, M.S., et al. *Leucasaspera*: A review, *PharmacognRev*.**4**(7),85--87, 2010.
20. Mazaz,Q.A., et al. The miracle plant(*Kalanchoepinnata*): a phytochemical and pharmacological review, *IJRAP***2**(5), 1478--1482, 2011.
21. Parsons, W.T.&Cuthbertson, E.G.*Noxious weeds of Australia*, Inkata Press, Melbourne, Australia, 692, 1992.
22. Ahmad, H., et al. *Mimosa pudica* L. (Laajvanti): An overview, *Pharmacogn Rev*. **6**(12), 115--124, 2012.

23. Ahmed, M., et al. Analgesic, diuretic, and anti-inflammatory principle from *Scopariadulcis*, *Pharmazie***56**, 657--660, 2001.

24. Warrier, P.K.,& Nair R.V. *Indian medicinal plants : a compendium of 500 species,* Orient Longman, Madras, 224, 2002.

25. Dasgupta, P., et al. *Averrhoacarambola*: An updated review, *International Journal of Pharma Research &Review***2**(7), 54--63, 2013.

26. Kumar, S., et al. Agro-history, uses, ecology and distribution of henna (*Lawsoniainermis* L. syn. L. alba Lam.). in*Henna: Cultivation, Improvement, and Trade*, Jodhpur, India: Central Arid Zone Research Institute, 2005, 11—12.

27. Zumrutdal, E., &Ozaslan M. A Miracle Plant for the Herbal Pharmacy; Henna (*Lawsoniainermis*), *International Journal of Pharmacology***8**,483--489, 2012.

28. Bhowmik, D., et al. Recent trends in indian traditional herbs *Syzygiumaromaticum* and its health benefits, *Journal of Pharmacognosy and Phytochemistry***1**(1), 13--22, 2012.

29. Dhale, D., &Bhoi A.S. Pharmacognostic characterization and phytochemical screening of *Achyranthesaspera*Linn.,*Curr. Agri. Res.***1**(1), 51--57, 2013.

30. Srivastav, S., et al.*Achyranthesaspera*-An important medicinal plant: A review,*J. Nat. Prod. Plant Resour.***1**(1), 1--14, 2011.

31. Chin, W.Y.,&Keng, H.*An Illustrated Dictionary of Chinese Medicinal Herbs,* CRCS Publications, 1992.

32. Brickell C. *The Royal Horticultural Society A-Z Encyclopedia of Garden Plants*, Dorling Kindersley, London, 884—885, 1996.

33. Jena, J., & Gupta, A.K.*RicinusCommunis* Linn: A phytopharmacological review. *International Journal Of Pharmacy And Pharmaceutical Sciences***4**(4), 2012.

34. Ghosh, K., &Baghel, M.S. A pharmacognostical and physiochemical study of *Benincashispida* with Ayurvedic review, *IJRAP***2**(6), 1664--1668, 2011.

35. Venkateswarlu, J., &Rao, K. M. Inheritance of fruit colour in the*Solanumnigrum* complex, *Proceedings: Plant Sciences* **74**(3), 137--141, 1971

36. Jain, R., et al. *Solanumnigrum*: current perspectives on therapeutic properties, *Altern Med Rev.* **16**(1), 78—85, 2011.

37. Ahmad, J., et al.Screening of some Indian medicinal plants for their antimicrobial properties,*J Ethnopharmacol***62**, 183--193, 1998.

38. Dey, D. et al. Inhibition of insulin receptor gene expression and insulin signaling by fatty acid: interplay of PKC isoforms therein, *Cell PhysiolBiochem.***16**(4-6), 217--228, 2005.

39. Mosmann, T. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays, *J Immunol Methods* **65**, 55--63, 1983.

40. Lowry, O. H., et al. Protein measurement with the Folin phenol reagent, *J.Biol.Chem.***193**(1), 265--275, 1951.

41. Barma, P., et al. Nutritionally induced insulin resistance, *Current science* **90**(2), 188--194, 2006.

42. Shekhar, S., et al. Ectopic expression of amaranth seed storage albumin modulates photoassimilate transport and nutrient acquisition in sweetpotato, *Sci.Rep.***6,** 1--14**,** 2016.

43. Shekhar, S., et al. Comparative analysis of phytochemicals and nutrient availability in two contrasting cultivars of sweet potato (*Ipomoea batatas* L.), *Food Chem.***173,** 957--965, 2015.

44. Dewanjee, S., et al. Swietenine: A potential oral hypoglycemic from Swieteniamacrophylla seed, *Fitoterapia* **80**(4), 249--251, 2009.

45. Jose, M., et al. The insulin signalling pathway, *Current biology* **12**(7), 236--238, 2002.