CHAPTER-II

REVIEW OF LITERATURE

2.1 Pharmacological investigation of plant materials

Review of literature involving research of medicinal plants for their phytological and pharmacological properties suggest that scientist's peruse more or less the same general strategy. Steps involved in this process are discussed below in addition with schematic diagram (Fig 1).

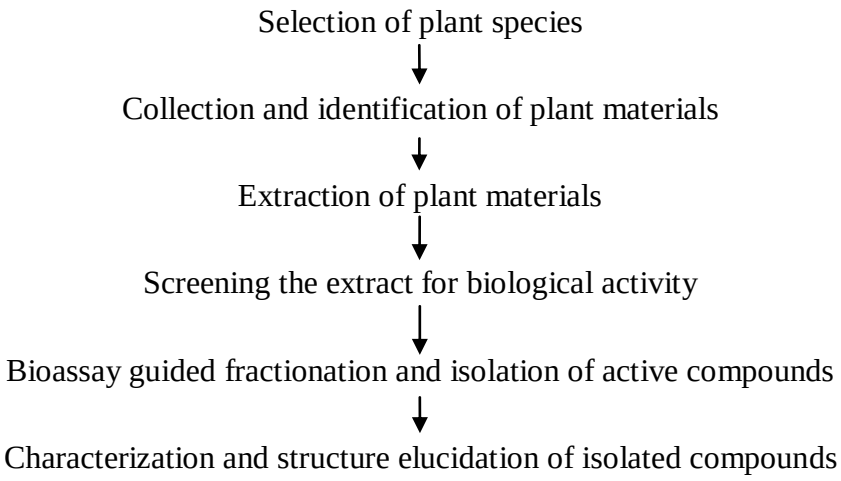


Fig 1: General strategy for pharmacological investigation of plant materials

2.1.1 Selection of plant species

All higher plants elaborate chemical secondary metabolites that are of potential medicinal interest. Therefore, the determination of criteria for selecting plants for phototherapeutic investigation is perhaps an important exercise as the investigation itself (Elizabeth *et al*., 1996). The plant material to be investigated can be selected on the basis of some specific traditional uses (ethnobotanical bioprospecting approach) as extract prepared from these plants are more likely to contain biologically active compounds of medicinal interest. Alternatively, the plant can be selected based on chemo taxonomical data. In the chemotaxonomic approach, knowledge that a particular group of plants contain a certain class of natural products may be used to predict that taxonomically related plant may contain structurally similar compounds. Some plant materials can be selected following a combination of the above mentioned approaches. The use of literature data base early in the selection process can provide some

preliminary information on the type of natural products already isolated from the plant and the extraction methods employed to isolate them (Heinrich *et al*., 2004). Another approach known as the information driven approach, utilizes a combination of ethobotanical, chemotaxonomic and random approaches together with a data base that contains all relevant information concerning a particular plant species (Kinghorn and Balandrin, 1993; Heinrich *et al*., 2004). The database is used to prioritize which plants should be extracted and screened for biological activity.

2.1.2 Collection and identification of plant materials

The whole plant or a particular part can be collected depending on where the metabolites of interest (if they are known) accumulate. Hence aerial (e.g. leaves, stems, flowering tops, fruit, seed, bark) and underground (e.g. tubers, bulbs, roots) parts can be collected separately. Collection of plant materials can be influenced by factors such as the age of the plant and environmental conditions (e.g. temperature, rainfall, amount of daylight, soil characteristics and altitude) (Williams *et al*., 1996). Thus, it is important to take this into consideration for the re-collection purpose, in order to ensure reproducible profile (nature and amount) of metabolites (Satyajit *et al*., 2006). The plant from which the material is collected must also be identified correctly. A plant taxonomist or a botanist should be involved in the detailed authentication of the plant (i.e. classification into its class, order, family, genus and species). Any feature related to the collection, such as the name of the plant, the identity of the parts collected, the place and date of collection, should be recorded as part of the voucher (a dried specimen pressed between sheets of paper) deposited in a herbarium for future reference.

2.1.3 Extraction of plant materials

Plant materials are commonly extracted by means of liquid solvents in what is known as the "solid-liquid solvent extraction". A typical solid-liquid solvent extraction process for plant materials involved drying and grinding of the plant materials, choosing a suitable extraction solvent followed by extraction procedure.

2.1.3.1 Drying and grinding

Once the plant material has been collected, it needs to be dried as soon as possible after washing wit tap water at ambient temperature with adequate ventilation to prevent microbial fermentation and subsequent degradation of metabolites. Plant materials should be sliced into small pieces and distributed evenly to facilitate homogeneous drying. Protection from direct sunlight is advised to minimize chemical reactions (and formation of artifacts) induced by ultraviolet rays. The dried material should be stored in sealed containers in a dry and cool place. Storage for prolonged periods should be avoided as some constituents may be decomposed (Heinrich *et al*., 2004; Jones and Kinghorn, 2005).

After drying, plant materials are commonly grounded into a fine powder. Grinding of plant materials into smaller particles facilitates subsequent extraction procedures by rendering the sample more homogeneous, increasing the surface area, and facilitating the penetration of solvents into cells. Mechanical grinders (e.g. hammer and cutting mills) are employed to shred the plant material into various particle sizes.

2.1.3.2 Choice of a suitable extraction solvent

The choice of the extraction solvent depends mainly on the polarity and hence the solubility of the bioactive compound(s) of interest. Although water is generally used as an extractant in many traditional protocols, organic solvents of varying polarities are often used (either alone or in different combinations) in modern methods of extraction to exploit the various solubilities of plant constituents. The polarity and chemical profiles of most of the common extraction solvents have been determined and are summarized below (Table 1).Thus, if the polarity or the solubility of the compound(s) of interest is known; information such as the one in the above table can be used to select a suitable extracting solvent or a mixture of two or more solvents of different polarity. If the polarity of the compounds of interest is not known, the powdered plant material can be extracted simultaneously with a mixture of different proportions of two or more solvents of different polarity. Alternatively, the powdered plant material can be extracted sequentially with solvent of different polarity in what is known as a sequential extraction procedure (Bruneton, 1999).

Polarity	Solvent	Extracted chemical profile	References
Low	n-Hexane, Chloroform	Fatty acids, waxes and terpenoids	Ayaffor *et al.*, 1994.Cowan,1999. Perett *et al*., 1995. Bruneton, 1999.
Medium	Dichloromethane, Ethyl-acetate	Less polar and polar flavonoids,tannins,terpenoids	Bruneton, 1999. Scalbert *et al*., 2005.
	Acetone	Less polar and polar flavonoids,tannins,terpenoids and glucosides	Eloff, 1998. Bruneton, 1999. Scalbert *et al*., 2005.
High	Ethanol	Polar liavonoids, tannins and glycosides (saponins)	Cowan, 1999. Bruneton, 1999.
	Methanol and water	Carbohydrates,lecitin, amino acids, polypeptides, phenolic acids,phenylpropanoids, polar flavanoids, glycosides and alkaloids	Bruneton, 1999. Scalbert *et al*., 2005. Kaul *et al.*, 1985. Jonesand Kinghorn, 2005.
	Aqueous acid and base	Alkaloids	Bruneton, 1999.

Table 1: Polarity and chemical profiles of most of the common extraction solvents

2.1.3.3 Choice of the extraction procedure

The choice of the extraction procedure depends on the nature of the source material and the compound to be isolated. Solvent extraction procedures applied to plant natural products include but not limited to maceration, percolation, soxhlet extraction, steam distillation and sequential solvent extraction (Starmans and Nijhuis, 1996; Jones and Kinghorn, 2005; Elizabeth *et al*., 1996).

Maceration: This is a simple process which involved soaking of the plant material in a suitable solvent, filtering and concentrating the extract. The advantage of this method is that it uses cold solvent, which reduces decomposition, but it takes longer and uses greater volume of solvent.

Percolation: This is similar to the maceration process, but hot solvent is refluxed through the plant material. It is quicker and uses less solvent, but decomposition due to heat may occur.

Soxhlet extraction: Soxhlet extraction is a form of continuous percolation with fresh solvent, which uses special glass ware. In this procedure, the plant material is separated from the extract by encasing it in a paper thimble beneath the dropping

condensed solvent. When full, the solvent in the thimble siphons off into the main vessel containing the extract, and the process continues. The advantage of this procedure is that fresh solvent continually extract the plant material more effectively with minimum solvent, however, heating is again a disadvantage.

Steam distillation: This is a special apparatus for distilling volatile oils which are immiscible with water. If compounds being extracted are water soluble, the method is less useful because a large volume of aqueous extract is produced. However, in some cases a partition system may be used to concentrate the extract.

Sequential solvent extraction: If the polarity and solubility of compounds that are isolated is not known, a convenient and frequently used procedure is sequential solvent extraction. In sequential solvent extraction, the plant material is extracted with a series of solvents of different polarity. The usual way is to start with a non-polar solvent and exhaustively extract the plant material followed by a series of more polar solvents until several extracts are obtained of increasing solute polarity. For example, a first step, with dichloromethane, will extract terperoids, less polar flavonoids and other less polar materials. A subsequent step with acetone or ethyl acetate will extract flavonoid glycosides and other medium polar constituents. A subsequent extraction with an alcohol or water will extract highly polar constituents.

Once the extraction is completed, the extract is usually concentrated under vacuum, for large volumes of solvents and blown down under nitrogen for small volumes, ensuring at the same time that volatiles are not lost. Aqueous extracts are generally freeze-dried and stored at $20^{o}C$ as this low temperature reduces the degradation of the bioactive natural product. Extraction protocols may sometimes be modified depending on the type of molecules being extracted, e.g. acid may be added to extract alkaloids as their salts.

2.1.4 Screening the extract for biological activity

Once the extract has been obtained, the biological activity within is usually demonstrated by means of an in vitro bioassay method. In vitro screening methods for biological activity are generally divided into two formats; the low-throughput screening and high-throughput screening methods, depending on the number of extracts to be

screened. In low-throughput screening (LTS), small numbers of extracts (a single to hundred of extracts) are dispensed into a format that is compatible with the bioassay (e.g. a microtitre plate or sample tube). This approach is used widely in academic laboratories where only a relatively low number of extracts are assessed. In high-throughput screening (HTS), thousand of extracts are dispensed into a format (usually a microtitre plate with many wells, e.g. 384 wells per plate) and screened in the bioassay. This approach is favored by the pharmaceutical industry. This may have hundreds of thousands of samples (both natural and synthetic) for biological evaluation. This large scale approach means that decisions can be made rapidly about the status of an extract, which has an impact on the cost of the drug discovery process (Kinghorn and Balandrin, 1993; Heinrich *et al*., 2004).

2.1.5 Bioassay guided fractionation and isolation of active compounds

Active fractions are fractionated using a bioassay guided fractionation. In bioassay-guided fractionation, a crude mixture is fractionated into its fraction components using chromatographic procedures, followed by biological evaluation (bioassay) of each fraction. Only fractions which display biological activity in the bioassay are selected for further fractionation. The cycle of fractionation and testing and further fractionation is repeated until a pure compound with the desired activity is isolated (Rimando *et al*., 2001). The general scheme for carrying out a bioassay guided fractionation is summarized below (Fig 2).

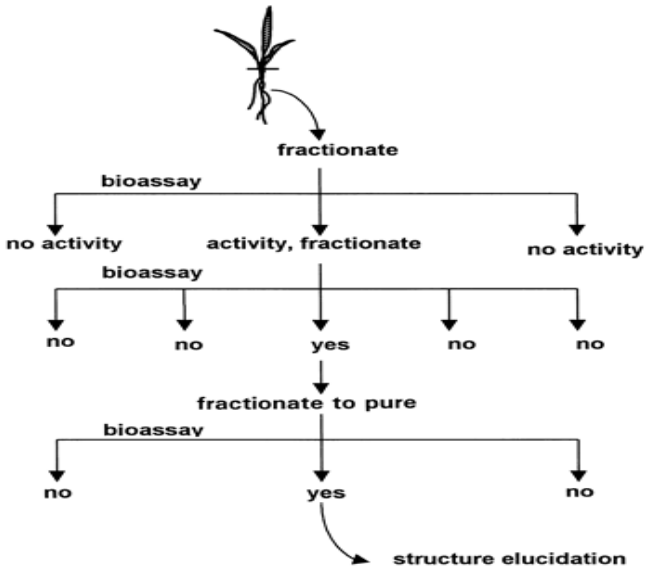


Fig 2: General scheme for bioassay-guided isolation of active compounds.

2.1.6 Characterization and structure elucidation of isolated compounds

Once the biological evaluation has been performed and the separation of the natural product has been achieved, the last attempt is the elucidation of the compound. Structure elucidation depends on classical spectroscopic techniques such as: Nuclear Magnetic Resonance (NMR) 1-D and 2-D Proton NMR as well as C-13 NMR, Infra Red (IR), Mass Spectrometry (MS) and X-Ray analysis.

2.2 Role of medicinal plants against diabetes

Currently, medicinal plants used to play a vital role in the management of diabetes mellitus, especially in developing countries. Regardless of considerable progress in the treatment of diabetes by oral hypoglycemic agents, search for newer drugs continues because the existing synthetic drugs have several limitations; side effects, high secondary failure rate and the cost of conventional synthetic antidiabetic remedies. For centuries plants have been used to treat human diseases, it also formed the basis of sophisticated traditional medicines systems such as Ayurvedic, Unani (Wadkar, 2008). India and China are two of the largest countries of Asia, which have the riches collection of registered and relatively well known medicinal plants (Raven, 1998). According to World Health Organization (WHO) more than 80% world population relies on traditional medicines derived from medicinal plants Also defined traditional medicine (including herbal drugs) as comprising therapeutic practices that have been in existence, almost for several hundreds of years, before the development and spread of modern medicine and others which are still in use (Modak *et al*., 2007). Therefore, collection of information and documentation of traditional knowledge plays an important role in scientific research on drug development (Ragupathy, 2008). India only has officially recorded a list of 45,000 plant species and a various estimation of 7500 species of medicinal importance (Ashis, 2005). India is the largest producer of medicinal herbs and is called as botanical garden of the world (Seth and Sharma, 2004).

Ethnobotanical surveys indicate that more than 1200 plants are used in traditional medical systems for their suspected hypoglycemic activity (Marles and Farnsworth, 1995). The hypoglycemic activity of a large number of these plants/plant products has been evaluated and confirmed in animal models as well as in human beings (Gupta *et al*., 2005). Earlier the plants have been used as crude extracts which

consisted of numerous active compounds. Some of these compounds may act synergistically, while at times they can have antagonist effects. Lately it has been focused on ethnobotany and ethnopharmacognosy in which many bioactive compounds were isolated and characterized. Among these are alkaloids, flavanoids, glyclipids, glycosides, polysaccharides, peptidoglycans, hypoglycans, galactomannans, guanidine, steroids, carbohydrates, glycopeptides, terpenoids, amino acids, saponins, dietary fibers and inorganic ions. Even the discovery of widely used hypoglycemic drug, metformin came from the traditional approach of using *Galega officinalis* (Gurub-Fakim, 2006). Thus, plants are a potential source of anti-diabetic drugs however there is a major hindrance in amalgamation of herbal medicine in modern medical practices due to lack of scientific and clinical data proving their efficacy and safety.

2.2.1 Most studied and commonly used antidiabetic medicinal plants

Medicinal plants play an important role in the management of diabetes mellitus and it has been confirmed by several methods using both in vitro and in vivo. Many studies have confirmed the benefits of medicinal plants with hypoglycemic effects in the management of diabetes mellitus thereby delaying the development of complications and correcting the metabolic abnormalities. Some of the medicinal plants which are commonly studied and used for antihyperglycemic effects include: *Allium cepa, Allium sativum, Momordica charantia, Murrayi koningii, Ocimum sanctum, Panax ginseng, Trigonella foenum-graecum, Ptericarpus marsupium and Syzigium cumini* (Bnouham *et al*., 2006). Plants extract used, active principles isolated, as well as the possible mechanism of action for the above mentioned medicinal plants and herbs in addition with others are listed below in Table 2.

Plant and Family	Plant part used	Active ingredient	Mechanism of action	References
Allium cepa (Onion) Alliaceae	Onion bulbs	S-methyl cysteine sulphoxide S-allylcysteine sulphoxide	Stimulate insulin secretion Compete with insulin for insulin-inactivating sites in the liver	Sheela *et al*., (1995) Kumari *et al*., (1995) Eidi *et al*., (2006)
Allium sativum (Garlic), Alliaceae	Garlic gloves	S-methyl cysteine sulphoxide-precursor of allicin and garlic oil	Stimulate in vitro insulin secretion.Inhibit glucose production by the liver	Sheela *et al*., (1992) Augusti and Shella (1996)
Aloe vera (*Aloe barbadensis*) Aspholedeceae	Leaf pulp and gel	Phytosterols	Stimulate synthesis and/or release of insulin Alter activity of carbohydrate metabolizing enzymes	Rajasekaran *et al*., (2004) Tanaka *et al*., (2006)
Camellia sinensis (green	Seeds	Epigallocatechin gallate	Increase insulin secreation	Ayodhya *et al*., (2010)

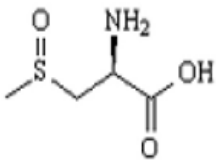
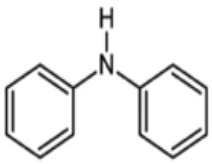
tea) Theaceae				
Catharanthus roseus (Madagascar periwinkle) Apocynaceae	Fresh leaf juice	Alkaloids: catharanthine, leurosine and vindolinine Tannins	Increases hepatic utilization of glucose Supress activities of gluconeogenic enzymes	Benjamin *et al*., (1994) Marles & Farnsworth, (1995) Singh *et al*., (2001) Nammi *et al*., (2003)
Cinnamomum cassia (Chinese cinnamon) Lauraceae	Bark	Cinnamaldehyde Cinnamic alcohol Methyl hydroxyl chaconne polymer	Enhances insulin action Increase glucose uptake and glycogen synthesis	Chase and McQeen, (2007)
Coccinia indica Cucurbitaceae	Leaves	Beta sitosterol	Suppress glucose 6-phosphatase Stimulate glycogen synthase activity and reduction of phosphorylase activity	Hossain *et al.*, (1992) Kumar *et al*., (1993))
Eugenia jambolina Mytaceae	Seeds	Pandanus odorus (Toei-hom) a 4-hydroxybenzoic acid	Enhances insulin secretion	Modak *et al*., (2007)
Fiscus bengalensis Moraceae	Leaves and bark	Lecoperlargonin derivative Leucocyandin	Increases insulin secretion Inhibit insulinase activity Blood sugar lowering activity	Achrecker *et al*., (1991) Augusti *et al*., (1994) Bnouham *et al*., (2006)
Gymnema Sylvestre (Gurmar). Asclepiadaceae	Leaves	Gymnemosides and gymnemic acid (from the saponin fraction) Triterpene glycosides	Stimulate insulin secretion from rat islets. Decreases the activity of gluconeogenic enzymes Induce beta cell regeneration	Shanmugasundaram, (1990) Chattopadhyay, (1999)
Ginseng (*Panax ginseng*) Araliaceae	Root and leaves	Polysacharides Ginsenosides (steroidal saponins)	Slow digestion and absorption of CHO Affect NO mediated glucose transport	Yang *et al*., (1990) Petit *et al*., (1995)
Mormordica charrantia (Bitter melon) Cucurbitaceae	Fruit pulp, seed, leaves and whole plant	Charantin (a peptide) Insulin like polypeptide P ("vegetable insulin")	Stimulatie insulin secretion Supress the activities of gluconeogenic enzymes Increases the number of beta cells in diabetic rats	Rao *et al*., (1999) Day *et al*., (1990) Sarkar *et al*., (1996
Murrayi koningii (Curry leaf) Rutaceae	Leaves	Carbazole alkaloids Copolin-a-glucoside	Stimulate insulin secretion ncreases glycogenesis and decrease glycogenolysis and gluconeogenesis	Kesari *et al*., (2005) Kesari *et al*., (2006)
Ocimum sanctum (Holy basil) Lamiaceae	Leaves	Pectins	Stimulate insulin secretion	Chattopadhyay,(1993) Agrawal *et al*., (1996) Rai *et al*., (1997) Vats *et al*., (2002)
Opuntia streptacantha (Cittrus colynthis) Cactaceae	Fruit	Saponins and glycosidic components	Stimulated insulin release from isolated pancreatic islets	Frati *et al*., (1991) Grover *et al*., (2002)
Polygala senega Polygalaceae	Rhizomes	Triterpenoid glycosides (Senegin II)	Increases insulin sensitiviry	Kako *et al*., (1996) Bnouham *et al*., (2006)
Polygonatum officinale Liaceae	Rhizomes	Unknown	Decreased hepatic glucose output Increased insulin sensitivity	Kako *et al*., (1996) Bnouham *et al*., (2006)
Psidium guajava Mrytaceae	Seeds	Flavonoid glycoside (Strictinin, isostrictinin and	Improved sensitivity of insulin	Chauhan *et al*., (2010)

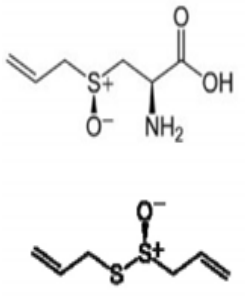
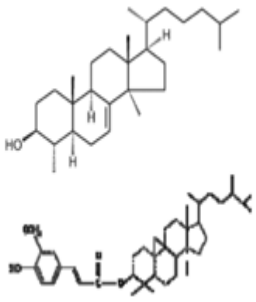
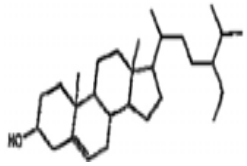
		pedunculagin)		
Pterocarpus marsupium Fabaceae	Bark	Epicatechin and catechin (tannin) Pterostilbene (flavonoid)	Prevent beta-cell damage in rats Regenerate functional pancre-atic beta cells Enhances insulin release	Manickam *et al*., (1997) Grover *et al*., (2002)
Stevia rabaudiana Asteraceae	Leaves	Glycoside stevioside	Stimulate insulin secretion via direct action on the β cells	Jung *et al.*, (2006)
Syzigium cumini (Eugenia jambolana) Myrtaceae	Seeds, leaves and fruit pulp	Mycaminose	Stimulate kinases involved in peripheral utilization of glucose	Achrekar *et al*., (1991) Kumar *et al*., (2008)
Trigonella foenum graecum (Fenugreek) Fabaceae	Seeds	Alkaloid-trigonelline, nicotinic acid, and coumarin 4-hydroxyisoleucine Galactomannan	Slow down digestion and absorption of CHO Increase glucose induced insulin release	Khosla *et al*., (1995) Hannan *et al*., (2007)

Table 2: List of commonly used antidiabetic plants.

2.3 Indian medicinal plants with confirmed antidiabetic activity

In India indigenous remedies have been used in the treatment of diabetes mellitus since the time of Charaka and Sushruta. However due to lack of clinical and pharmacological studies unlike synthetic molecules, little work has been done on the isolation and characterization of the phytocompounds therefore it is lacking much behind to the exploitation of the potential of these plant molecules. Details of some potent Indian herbs, their recently reported pharmacological and clinical hypoglycemic efficacy, active chemical constituents, their mechanism of action and available toxicity status are summarized in Table 3.

Plant and Family	**Part used**	**Structure of the active phytoconstituents having anti-diabetic potential/s**	**Pharmacological activity as antidiabetic**	**Dose**	**Model used**	**Reference**
Allium cepa L. (onion) Family: Liliaceae	Bulb	 	S-methyl cysteine sulfoxide (SMCS) showed antidiabetic and hyperlipidemic activity • Anti-hyperglycemic and anti-hyperlipidemic activity • Anti-hyperglycemic and insulin resistance in high fat diet	200 mg/kg body weight (BW) of SMCS 200 mg/kg BW of SMCS 2% freeze dried powder	Alloxanized rats High cholesterol diet-fed rats STZ rats	Kumari *et al.*, (1995). Islam *et al.*, (2008). Kumari and Augusti, (2002). Karawya et al.,(1984).

Allium sativum Linn. (Family: Alliaceae)	Clove		S-allyl cysteine (SACS) showed beneficial effect on antioxidant system • SACS showed anti-diabetic activity • Allicin lowered the blood pressure and improved lipid profile in hyperlipidemic, hyperinsulinemic • Anti-diabetic activity	150 mg/kg BW of SACS 200 mg/kg BW of SACS 8 mg/kg BW of allicin 0.5 mg/kg BW of ethanolic extract	STZ rats Alloxanized rats Fructoseinduced hyperinsulinemic hyperlipedemic, hypertensive rats STZ rats	Augusti and Sheela, (1996). Mathew and Augusti, (1973). Elkayam, (2003).
Aloe vera (L.) Burm.f. (aloe) Family: Aloaceae	Leaf		Anti-hyperglycemic activity with protective effect on pancreas, liver and small intestine • Hypoglycemic effect of aloe • Hypoglycemic activity • Hypoglycemic and reduced HbA1c	300 mg/kg BW of ethanolic extract 500 mg/kg BW of dried sap 300 mg/kg BW of ethanolic extract 300 mg/kg BW of Ethanolic extract	STZ rats Alloxanized mice STZ rats Alloxanized rabbits	Tanaka, (2006).
Azadirachta indica A. Juss. (neem) Family: Meliaceae	Leaf and seeds		Hypoglycemic activity • Hypoglycemic and restricted oxidative stress • Anti-hyperglycemic activity • Reduced intestinal glucosidase activity and anti-hyperglycemic properties	Hydro alcoholic extract 2 mg/kg BW of petroleum ether extract of seed kernel 250 mg/kg BW of crude ethanol extract 100 µg of chloroform leaf extract	STZ rats STZ rats Alloxanized rabbits STZ mice	Prabhakar and Doble, (2008).

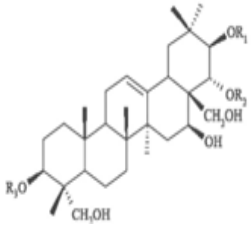
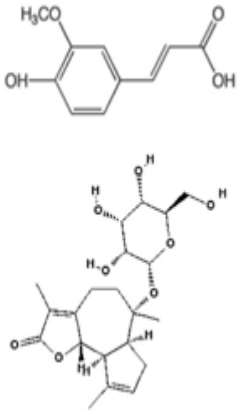
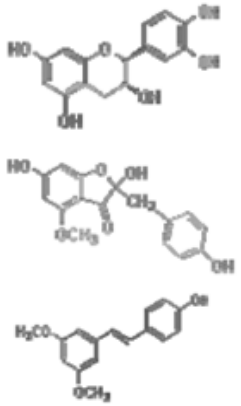
Gymnema sylvestre (Periploca of the woods) Family: Ascelpiadaceae	Leaf		Anti-diabetic activity • Anti-hyperglycemic effect • Hypolipidemic effect in hypertensive rats	200 mg/kg BW of methanol extract Powdered leaves 1.6% w/w of 25% gymnemic acid content	Alloxanized rats Beryllium nitrate-treated rats Spontaneously hypertensive rats	Sugiraha *et al*., (2000). Kimura, (2006).
Syzygium cumini Walp. (Eugenia jambolana) (blackberry) Family: Myrtaceae	Seed and pulp		Hypoglycemic and anti-oxidant activity • Hypoglycemic activity • Anti-hyperglycemic effect • α-Glucosidase inhibitory activity	2.5 and 5 g/kg BW of aqueous seed extract 500 mg/kg BW of seed powder 25 mg/kg BW of water and ethanolic extract of fruit pulp 250 mg/kg BW of seed kernel acetone extract	Alloxanized rats STZ rats Alloxanized rabbits Goto–Kakizaki rats	Mandal *et al*., (2008). Farswan, (2008).
Pterocarpus marsupium Roxb. (Indian kino tree) Family: Fabaceae	Bark		Anti-diabetic and protective effect on serum protein, ALP and ACP, albumin levels and HbA1c • Anti-hyperglycemic activity • Hypoglycemic activity • Hepatoprotective effect	300 mg/kg of methanolic extract 0.25 g/kg BW of ethanol extract 250 mg/kg BW of aqueous extract 25 mg/kg of methanol extract	STZ rats STZ rats Alloxanized rats Wistar rats	Sheehan *et al*., (1983). Manickam et al., (1997).

Table 3: Indian medicinal plants with confirmed antidiabetic activity.

2.4 *Eugenia operculata* Roxb.

2.4.1 Classification

Kingdom: Plantae
Division: Magnoliophyta
Class: Magnoliopsida
Order: Myrtales
Genus: *Eugenia*
Family: Myrtaceae
Species: *operculata*

Plate 1: *Eugenia operculata* Roxb.

2.4.2 Description

Eugenia operculata Roxb. 1832, is also known as *Cleistocalyx operculatus, Cleistocalyx nervosum, Syzygium nervosum*. It is a small or medium sized evergreen tree, upto 10 meter in height with pale brown or grey bark with exfoliating in irregular flakes. Leaves thinly coriaceous, broadly ovate or elliptic, rarely obovate, glabrous, apex rounded or obtusely acuminate, margin entire, base narrowed measuring 7-9 cm in length. Flower cluster as greenish white, small sessile, odorous, trichomatous panicles in leafless axils. The blossoms have four petals. Fruits are berry dark purple, ovoid or globous with a concave tip and a wrinkled texture, 0.6-1.2 cm in diameter and juicy, crowned by calyx rim.

2.4.3 Distribution and habitat

In India Eugenia operculata Roxb has found distributed within Sub-Himalayan forests, tract and also in Bihar, Orissa, Assam and Manipur.

2.4.4 Phytochemical studies

Previous phytochemical attention has led to the characterization of oleanane type triterpene from its bark (Nomura *et al.*, 1993) and flavanone, triterpic acid, sterol, chalcone, β sterol and ursolic acid as the main constituent in the methanol extract of buds (Zhang *et al*., 1990). The ethanol extract from the seed were found to contain phenolic and flavanoid (Ye *et al*., 2004).

2.4.5 Uses of *Eugenia operculata* Roxb.

The leaves and buds of *E.operculata* have been used as an ingredient in various beverages, common tea for gastrointestinal disorders and as an antisepsis for dermatophytic disorders for many years (Loi, 1986). The fruit is eaten for rhumentism. A concentrated of the root infusion is used against painful joints. The bark is acrid, bitter and astringent and is given in dysentery, bronchitis and biliousness. Previous reports also revealed that the *E.operculata* buds had various biological activities in vitro and in vivo such as anticancer, antitumor, antihyperglycemic and cardio tonic action (Loi, 1986; Ye *et al*., 2005a,b; Mai *et al*., 2007; Anthony *et al*., 2008). High contents of polyphenols and flavonoids in *E.operculata* were known to have antioxidant and anticarcinogenic properties also it stimulated human lymphocyte proliferative responses and significantly enhanced NK cells activity (Sriwanthana *et al*., 2007). The stem bark is used for ritual and religious in North-Eastern parts of India. In folk medicine, the ash of dried bark is given in a dose of 1.25g with water on empty stomach or 1hr before lunch and dinner for 40 days to diabetic patients.

2.4.6 Hypoglycemic properties of *E.operculata*

The blood glucose lowering effect of *E.o* extracts have been studied and confirmed using experimental animal models of diabetes. Aqueous extract of *E.o* flower buds inhibited the rat intestinal α glucosidase, maltase and sucrase activities, with IC_{50} values of 0.70 and 0.47mg/ml respectively, inhibition of the activity of

carbohydrates hydrolyzing enzymes plays an important role in the prevention and treatment of diabetes. Again prolonged administration of *E.o* extract at a dosage of 500mg/kg b.w for 8 weeks to STZ induced diabetic rats clarified more antihyperglycemic effects viz restoration of the weight gain, reduction of blood glucose and urine volume (Mai and Chuyen, 2007).