REVIEW OF LITERATURE
3. REVIEW OF LITERATURE

3.1 Introduction to traditional system of medicine

From the time immemorial, man has depended on plants as medicine. From a historical perspective, it is evident that the fascination with plants is as old as mankind itself. Herbs have provided us some of the very important life saving drugs used in the armamentarium of modern medicine. The plant kingdom represents a rich storehouse of organic compounds, many of which have been used for medicinal and other purpose. There exists a plethora of knowledge and information and benefits of herbal drugs in our ancient literature of Ayurvedic and Unani medicine. Ethnomedicines are replete with descriptions of plant medicines and the grandmothers pouch that has been called for years of medical wisdom is excellent proof of efficacy of these experimental medicines.

3.1.1 Ayurveda

Ayurveda is one of the major traditional medicinal systems from India. It is presumed that the knowledge of Ayurveda is given by Gods of different world. It is accepted as the oldest written medical system. The word “Ayurveda” means “science of life”. It is the ancient Indian system of health care and longevity. Ayurveda takes a holistic view of man, his health and illness. It aims at a positive health, which has been defined as a well balanced metabolism coupled with a healthy state of being. Disease, according to Ayurveda, can arise from body and/or mind due to external factors or intrinsic causes. Ayurvedic treatment consists of salubrious use of drugs, diets and certain practices. The basic concept of diagnosis and drug development in Ayurveda is based on Tridosha (three major components of disorders) theory, which includes Vayu, Pitta and Kapha. Vayu (Vata) it explains the entire biological phenomenon, which are controlled by the functions of central and autonomous nervous system. Pitta: It is manifestation of energy (Tejas) in the living organisms that helps digestion, assimilation, tissue building, heat production, blood pigmentation, activities of endocrine glands and so on. Kapha: It implies the function of thermotaxis or heat regulation and also the formation of various preservative fluids e.g. mucus, sinovial fluid etc.
Ayurveda has a vast literature in Sanskrit and in various Indian languages, covering all aspects of diseases, therapeutics and pharmacy. Pharmaceutics occupies an important place in Ayurveda. Medicinal preparations are invariably complex mixtures, derived from plant and animal products as also minerals and metals forming a dominant part. Earliest references to such plants are found in two holy books Rigveda and Atharva Veda, dating back to second millennium BC. The Ayurveda is said to be an Upaveda (part of Atharva Veda) whereas, the Charaka Samhita (1900 BC) is the first recorded treatise fully devoted to concept and practice of Ayurveda. This describes 341 plants and plant products for use in medicine. The next landmark of the Ayurvedic literature was the Sushruta Samhita (600 BC), which has special emphasis on surgery. It has six sections covering 186 chapters, and describes 395 medicinal plants, 57 drugs of animal origin and 64 minerals and metals as therapeutic agents. With the introduction of Western scientific methods in India, many Ayurvedic drugs and other Indian plants with curative properties soon came under some sort of scrutiny. Such investigations have continued to the present day and are being reviewed continuously.

3.1.2 Siddha System of Medicine

The term Siddha comes from 'Siddhi' means attainment of perfection. This system is almost akin to Ayurveda. This system describes 96 principal constituents of human beings, which include physical, physiological, moral and intellectual components of individuals. When there is any imbalance or slight deviation with these 96 units, diseases occurs. The Siddha medicine consists of psychosomatic system where attention is given to minerals and metals rather than plant constituents. The use of metals and minerals form an integral part of Siddha system of therapy to cure diseases.

There are similarities between Siddha and Ayurveda in their basic principles based on the theory of panchamahabhuta meaning that everything in the world and the universe around it are made up of five basic elements – earth, water, fire, air and space. Detoxification is a common phenomenon for any given drug to increase their therapeutic potency thereby minimizing the toxicity, known as Suddhi Seithal.
3.1.3 Unani System of Medicine

Unani medicine owes its origin to Greece. In this system, diseases are considered as a natural process and its symptoms are the reaction of the body to the diseases. Unani system based on humoral theory, there are several humors in the body like Dam (Blood), Bhalgham (Phlegm), Safra (Yellow bile), and Souda (Black bile). Unani system believes that every person has a unique humoral constitution, which represents his healthy state. Any change in his state affects his health. There is a power of self-preservation or adjustment called ‘medicatrix naturae’ or defense mechanism, which strive to restore disturbances. If this power weakens, imbalance in humoral composition occurs and causes diseases. The medicines help the body to regain this power to an optimum level and thereby restore humoral balance and thus retaining health.

Various types of treatments are prescribed in Unani system of medicine. There are:

(a) Regimental therapy: includes Deaphoresis, Diuresis, Turkish bath, massage, emesis, purging etc.

(b) Dieto therapy: aims at treating certain ailments by administration of specific diets or by regulating the quantity and quality of food.

(c) Pharmacotherapy: deals with the use of naturally occurring drugs mostly herbal.

3.1.4 Homeopathic Remedies

Homeopathy is based on the idea that “like cures like”; that is, substances that cause certain symptoms in a health person can also cure those same symptoms in someone who is sick. This so-called law of similar gives homeopathy its name.

3.1.5 Tibetan System

The 7th and 8th century AD observed the real development in the field of Tibetan medicine. Ayurveda has contributed a great deal in enriching Tibetan medicine. Pulse diagnosis and urine analyses form the distinctive features of Tibetan medical system. The fundamental concepts of Tibetan medical systems, like Ayurveda and Siddha revolves around the five cosmo-physical energies and tree humoral energies. The working concepts of Ayurveda like panchabhaustica, tri-doshas, saptat dhutas, malatraya etc. also constitute the main features of Tibetan medicine. The most distinctive feature is its
integrated Buddhist approach to mind and body relationship, the application of ‘marigpa’ or ignorance, and the three inborn mental poisons like attachment, anger and delusions being the main cause of all suffering.

3.2 Plants and treatment of Diabetes Mellitus

Traditional medicines for the treatment of diabetes mellitus are probably based mainly on treatment of its obvious symptoms of pronounced thirst and polyuria. Even glucosuria was recognized as a symptom of diabetes in ancient Ayurvedic medical texts such as the Sushruta Samhita and Charaka Samhita (Nagarajan et al., 1982). The Greek physician Aretaeus recommended treatment of diabetes by treatment of profound thirst. For this he recommended starting with a purgative to strengthen the stomach, followed by consuming water boiled with autumn fruit a good source of soluble fibre and complex carbohydrates like pectin, milk, gruels of a variety of whole grains (an excellent source of soluble and insoluble fibre and glycans) and astringent wines (Hengesh and Holcomb 1981; Lomeo et al., 1988). He also recommended a crude drug of animal origins: venom of the “dipsas” viper, which in bite victims causes a severe thirst. Aretaeus suggested it could be used as a mithridate, i.e., a poison which is deliberately administered in small, gradually increasing doses in order to develop immunity to the effect of the poison (Adams 1856). In fact, the venom of the Middle Eastern viper Piscivorus piscivorus (crotalidae) was found to be hypoglycemic when administered i.v. at a dose of 10 μg/kg in normal rats and rabbits, but was inactive against alloxan induced hyperglycemia in rats (Taha 1982).

More than 1200 species of organisms have been used ethnopharmacologically or experimentally to treat symptoms of diabetes mellitus. They represent more than 725 genera in 183 families, extending phylogenetically all the way from marine algae and fungi to advanced plants such as the composites. The most frequently cited families are shown in Table 1. They are very large and widely distributed families, so the large number of species reported to have been used traditionally or experimentally for the treatment of diabetes may be coincidental. The phylogenetic distance between even these select groups of families is a strong indication of the varied nature of the active constituents. Thus, chemotaxonomic studies are often useful in the discovery of new
plants with biologically active constituent. It will be necessary to learn more about particular groups of hypoglycemic natural products and their mechanism action before this method of drug discovery can be successfully employed.

**Table 1. Plants family more often cited for antidiabetic activity.**

<table>
<thead>
<tr>
<th>Family</th>
<th>Species cited for antidiabetic activity</th>
<th>Total species</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fabaceae</td>
<td>127</td>
<td>18,000</td>
</tr>
<tr>
<td>Asteraceae</td>
<td>98</td>
<td>21,000</td>
</tr>
<tr>
<td>Lamtaceae</td>
<td>36</td>
<td>3,500</td>
</tr>
<tr>
<td>Liliaceae</td>
<td>35</td>
<td>6,460</td>
</tr>
<tr>
<td>Poaceae</td>
<td>30</td>
<td>11,000</td>
</tr>
<tr>
<td>Euphorbiaceae</td>
<td>30</td>
<td>7,000</td>
</tr>
</tbody>
</table>

According Throne et al., 1981.

Half of the species found in our literature review have been used in traditional medicine to treat the symptoms of diabetes. Half of these traditional medicines have some experimental testing for hypoglycemic activity, e.g., in normal, glucose-loaded, alloxan or streptozotocin induced diabetic or naturally diabetic subjects. Distinctions of the experimental model used are clearly important for gaining and understanding of the mechanism of action of the botanical drugs.

A summary of the results of screens for blood glucose lowering activity, presented in Table 2, shows that 81% of those traditional antidiabetic plants tested gave positive results. Even for those plants which no traditional use was mentioned, 47% of those species screened were active. This rate of positive results is higher than one would except by random chance—perhaps 10% would be reasonable, based on the number of active species obtained by the U.S. National Cancer Institute’s random screening of more than 35,000 species for antitumor activity. The high percentage of active plants probably reflects, at least in part, the great variety of possible active constituents and mechanisms
of action. Study of traditional remedies for diabetes mellitus yields an excellent return in potential new sources of antidiabetic drugs.

Table 2. Activity of traditional antidiabetic vs. other plants

<table>
<thead>
<tr>
<th></th>
<th>Traditional</th>
<th>Others</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total no. tested</td>
<td>295*</td>
<td>541</td>
</tr>
<tr>
<td>Total active</td>
<td>238(81%)</td>
<td>254(47%)</td>
</tr>
</tbody>
</table>

* Out of a total of 582 known traditionally used plants

Table 3. Most widely used traditional antidiabetic plants

<table>
<thead>
<tr>
<th>Scientific name</th>
<th>Countries where used traditionally</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cucurbitaceae</td>
<td>Saudi Arabia, West Africa, Pakistan, India, Thailand, England</td>
</tr>
<tr>
<td><em>Momordica chirantia</em></td>
<td></td>
</tr>
<tr>
<td>Apocynaceae</td>
<td>Australia, England, India, Thailand, Vietnam, Mozambique</td>
</tr>
<tr>
<td><em>Catheranthus roseus</em></td>
<td></td>
</tr>
<tr>
<td>Anacardeaeae</td>
<td>Ecuador, Colombia, Mexico, India, Madagascar, Thailand, England</td>
</tr>
<tr>
<td><em>Anacardium oceidentale</em></td>
<td></td>
</tr>
<tr>
<td>Myrtaceae</td>
<td>Pakistan, India, Thailand, West Indies, West Indies, Mexico, China, Guatemala</td>
</tr>
<tr>
<td><em>Syzygium cumini</em></td>
<td></td>
</tr>
<tr>
<td>Eucalyptus globules</td>
<td></td>
</tr>
<tr>
<td>Fabaceae</td>
<td>Canary Islands, India, Israel, Morocco, Israel, Egypt, India, France</td>
</tr>
<tr>
<td><em>Lupinus albus</em></td>
<td></td>
</tr>
<tr>
<td>Trigonella foenum-graceum</td>
<td></td>
</tr>
<tr>
<td>Liliaceae</td>
<td>Haiti, India, Tunisia, Kuwait, India, Saudi Arabia, North Africa, Peru, India, Saudi Arabia, Mexico, Venezuela</td>
</tr>
<tr>
<td><em>Aloe vera</em></td>
<td></td>
</tr>
<tr>
<td><em>Allium cepa</em></td>
<td></td>
</tr>
<tr>
<td><em>Allium sativum</em></td>
<td></td>
</tr>
</tbody>
</table>
Review of literature

<table>
<thead>
<tr>
<th>Family</th>
<th>Species</th>
<th>Distribution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bignoneaceae</td>
<td>Tecoma stans</td>
<td>India, Mexico, Cuba, Guatemala</td>
</tr>
<tr>
<td>Urticaceae</td>
<td>Urtica dioica</td>
<td>Europe, USA, Nepal, India, Guatemala</td>
</tr>
<tr>
<td>Astraceae</td>
<td>Taraxacum officinale</td>
<td>Europe, Costa Rica, Mexico, USA</td>
</tr>
<tr>
<td>Cypraceae</td>
<td>Kyllinga monocephala</td>
<td>India, Indonesia, South Africa, Ethiopia</td>
</tr>
<tr>
<td>Euphorbiaceae</td>
<td>Phyllanthus amblica</td>
<td>India, Nepal, Tibet, Pakistan</td>
</tr>
<tr>
<td></td>
<td>Phyllanthus niruri</td>
<td>Indonesia, India, West Indies, Brazil</td>
</tr>
<tr>
<td>Melliaceae</td>
<td>Azadirachta indica</td>
<td>India, Fiji, Saudi Arabia, Trinidad</td>
</tr>
<tr>
<td>Moraceae</td>
<td>Morus alba</td>
<td>India, USSR, China, Peru</td>
</tr>
<tr>
<td>Rosaceae</td>
<td>Poterium ancistroides</td>
<td>Spain, Greece, Syria, Israel</td>
</tr>
<tr>
<td>Apiceae</td>
<td>Daucus carota</td>
<td>India, China, England, USA</td>
</tr>
</tbody>
</table>

If the same or closely related plants are used traditionally for the same purpose in more than one country, it suggests either cultural contact among the countries or independent discovery. In either case, the conservation of that traditional use indicates a higher probability that the traditional practitioners found the remedy to be effective. Table 3 give the list of twenty most widely used traditional antidiabetic plants. With the notable exception of Kyllinga, all of these species have already been studied and shown to be active or have active constituents, and for the most of them the identity of the probable active constituents is known. Several of these plants will be discussed in detail below.

Seventeen of the twenty most widely used traditional antidiabetic plants, and many others too, are in India. The Indian subcontinent has an extensive indigenous
Review of literature

pharmacopoeia, including the Ayurvedic, Unani, and Folkloric medical systems, which has already supplied the world with such useful drugs as reserpine, from *Rauwolfia serpentina*, which is used as an antihypertensive and tranquilizer (Tyler et al., 1981). Reserpine is also reported to be hypoglycemic in normal animals and animals made hyperglycemic by pretreatment with epinephrine (Ricci and Ricordati 1955). Indian traditional medicines may very well supply the world with some new antidiabetic drugs.

Several reviews of plants with known antidiabetic activity or traditional use as antidiabetic remedies have been published (Farnsworth and Segelman 1971; Ajgaonkar 1979; Oliver-Bever and Zahnd 1979; Oliver-Bever 1980; Nagarajan et al., 1982; Mossa 1985; Oliver-Bever 1986; Day and Bailey 1988; Bailey and Day 1989; Handa et al., 1989, Rahman and Zaman 1989; Ivorra et al., 1989; Winkelman 1989).

3.2.1 Hypoglycemic Constituents and Mechanisms of Action

To understand how plant constituents can be hypoglycemic in animals, it is worthwhile to consider the reasons why compounds with hypoglycemic activity occur in plants. In general, discussions of medicinal agents from plants center on plants secondary metabolites, i.e., non-ubiquitous constituents with no known essential role in the plant's metabolism.

It has been postulated that bioactive plant secondary metabolites may play a role in chemical defense mechanisms (Ehrlich and Reven 1964; Berenbaum 1983). While the precise mechanism that may be involved in chemically mediated coevolution between plants and herbivores or pathogenic organisms is controversial (Strong et al., 1984, Spencer 1988), it has been suggested that natural selection would ensure the survival for reproduction of those individuals of a species having the gene coding for production of a toxin, while individuals without the toxin would be consumed (Williams et al. 1989). Most hypoglycemic plant constituents, such as the *Catharanthus* alkaloids, might fit in this category, but there are other rather common plant constituents for which this explanation is not entirely satisfactory.

At the cellular and molecular levels, plants and animals are not very different in their metabolic processes. Glucose is the metabolic energy source and most important
biosynthetic precursor in plants, so glucose undergoes storage and mobilization under hormonal control in plants as it does in animals. Plant growth regulators such as indole-3-acetic acid (Fig 1, 1) and natural and synthetic analogs such as indole-3-butyric acid, indole-3-propionic acid, L-tryptophan (2), and p-chlorophenoxyacetic acid (3), inhibit insulinase in vitro and are hypoglycemic in vivo in normal rats (Mirsky et al. 1956). Nicotinic acid (4) and anthranilic acid (5) also inhibit insulinase and potentiate simultaneously administered insulin. An inhibitor of indole-3-acetic acid oxidase from Phaseolus vulgaris fruit exocarps also has hypoglycemic activity. The hypoglycemic alkaloid trigonelline (6) from Trigonella foenum-graecum, is a plant growth inhibitor and produces dormancy.

Salicylic acid (7) is also a plant growth inhibitor and hypoglycemic agent (Oliver-Bever and Zahnd 1979). Thus, plant metabolism-regulating constituents can also be animal metabolism-regulating agents. The variety of ways in which this may be possible will become clear with the discussion of hypoglycemic mechanisms of action to follow.

Possible active hypoglycemic constituents have been reported for 88 (16%) of the plants used traditionally as antidiabetics and 62 (11%) of the other plants screened. There
are more than 200 pure compounds from plant sources reported to show blood glucose lowering activity. Table 4 provides a summary of the chemical classes and indicates that a variety of mechanisms must be involved in the lowering of blood glucose level. Some of these compounds may have therapeutic potential, while others may produce hypoglycemia as a side-effect of their toxicity, especially hepatotoxicity.

### Table 4. Hypoglycemic Natural Products.

<table>
<thead>
<tr>
<th>Chemical class</th>
<th>Number active</th>
<th>Chemical class</th>
<th>Number active</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkaloids</td>
<td>38</td>
<td>Peptides &amp; amines</td>
<td>15</td>
</tr>
<tr>
<td>Carbohydrates</td>
<td>66</td>
<td>Phenolics</td>
<td>4</td>
</tr>
<tr>
<td>Coumarins</td>
<td>4</td>
<td>Phenolpropanoids</td>
<td>1</td>
</tr>
<tr>
<td>Cynogenic Glycosides</td>
<td>1</td>
<td>Steroids</td>
<td>7</td>
</tr>
<tr>
<td>Flavanoids</td>
<td>7</td>
<td>Stilbenes</td>
<td>1</td>
</tr>
<tr>
<td>Glycopeptides</td>
<td>20</td>
<td>Sulphur compounds</td>
<td>2</td>
</tr>
<tr>
<td>Inorganic Salts</td>
<td>3</td>
<td>Terpenoids</td>
<td>17</td>
</tr>
<tr>
<td>Iridoids</td>
<td>4</td>
<td>Vitamins</td>
<td>2</td>
</tr>
<tr>
<td>Lipids</td>
<td>6</td>
<td>Xanthenes</td>
<td>1</td>
</tr>
</tbody>
</table>

Some of the compounds reported to be active in vitro or at high doses in vivo, e.g., \(\beta\)-sitosterol-D-glucoside (Daucosterol, Fig 2, 9), occur so widely in nature that therapeutic activity seems unlikely. This could be due to their low concentration in the plants or co-occurrence with complexing or counteracting constituents. Some examples of plants with known active constituents and known mechanisms of action will be described below to show the range of active constituents and mechanism of hypoglycemic action.

#### 3.2.2 Peptides and Terpenoids from Momordica

The most widely used traditional remedy for diabetes mellitus is *Momordica chirantia* L. (Cucurbitaceae), common names for which are “bitter gourd,” “balsam pear,” “cundeamor,” and “cerasee.” The fruit, leaf, and stem have been used to make

Several active compounds have been isolated from *M. charantia* (Fig 2) and some mechanistic studies have been done. Khanna et al., (1981) have reported the isolation from the fruits, seeds and tissue culture of seedlings of “poly-peptide-p,” a 17-amino acid, 166-residue polypeptide which did not cross-react in an immunoassay for bovine insulin. This peptide was shown to be “insulinomimetic” when administered subcutaneously in rodent and primate experimental assays and in a limited clinical trial with both juvenile-and maturity – onset diabetic patients. A number of other polypeptides from *M. charantia* seeds have been studied in vitro for the insulin-like activities of stimulation of lipogenesis and inhibition of corticotropin-induced lipolysis. The mechanism was suggested to involve interaction of the peptides with α-adrenergic or corticotropin receptors (Ng et al., 1986).

Another active constituent, charantin, has been isolated from both *M. charantia* and *M. foetida*, and identified as a mixture of two steroid glycosides: β-sitosterol-D-glucoside (8) and 5,25-stigmastadien-3-β-ol-D-glucoside (9). Antihyperglycemic activity in alloxan-treated rabbits and depancreatized cats dosed p.o. or i.v. was equivocal, but hypoglycemic activity was observed in normal rabbits, rats, and cats dosed p.o., i.p., or i.v. (Lotlikar and Rajarama Rao 1966). Studies performed in vitro with *M. charantia* fruit extracts indicated a significant enhancement of glucose uptake in muscle tissue and of glycogen accumulation in muscle and hepatic tissue, but no effect on glucose uptake or triglyceride synthesis in adipose tissue (Meir and Yaniv 1985, Welihinda and Karunanayake 1986). Inhibition of glucose uptake by intestinal fragments was also observed and attributed to a glycosidic constituent of the fruit extract (Meir and Yaniv 1985). Thus, there appear to be constituents of *M. charantia* with both pancreatic and
extra pancreatic effects with therapeutic potential for diabetic patients. Caution is advised, however, because a mildly toxic lectin has been reported from the seeds and outer rind of the fruits, which is capable of interfering with protein synthesis in the intestinal wall (Lampe and McCann 1985).

3.2.3 Alkaloids from Catharanthus

The Madagascar periwinkle (*Catharanthus roseus* (L.) G. Don, Apocynaceae), is another widely used traditional remedy for diabetes and a proprietary preparation; Vinculin was marketed in England as a “treatment” for diabetes. Pharmacological studies have been conducted on periwinkles since the 1920’s. While two studies of leaf aqueous extracts administered orally to rabbits (Asthana and Misra 1979) and dogs (Morrison and West 1982) reported a hypoglycemic response, many other experiments with a variety of laboratory animals and limited clinical studies have given negative or at best equivocal results (Noble et al., 1958, Farnsworth 1961, Svoboda et al., 1959 and 1964, Farnsworth and Segelman 1971, Swanston-Flatt et al., 1989).

Despite these disappointing results, Svoboda et al. (1964) tested for hypoglycemic activity a number of alkaloids (Fig. 3) isolated from *C. roseus* during an investigation of the plant’s oncolytic activity, which was discovered by Noble et al. (1958) while investigating the plant’s reputed antidiabetic activity. Hypoglycemic activity was observed for catharanthine (10), leurosine (11), lochnerine (12), tetrahydroalstonine (13), vindoline (14), and vindolinine (15). Administered orally in a dose of 100 mg/kg,
leurosine sulfate and vindolinine hydrochloride were more hypoglycemic than tolbutamide, (Svoboda et al., 1964). Svoboda et al (1964) suggested that toxicity of crude extracts and fractions (e.g., several of the alkaloids are potent cytotoxic agents) may have made their experimental antidiabetic verification difficult, but that further study of C. roseus as a natural antidiabetic agent would be worthwhile. Some progress has been made in this direction. The Catharanthus and Vinca alkaloids, vincamine (16) and (-)-eburnamomine (17), have been shown to induce an extensive decrease in rat brain tissue glucose, with a concomitant increase in lactate and pyruvate concentrations and the lactate/pyruvate ratio. And an increase in ATP contents and energy charge potential (Benzi et al., 1984). Tetrahydroalstonine (13), administered orally in rats with alloxan-induced hyperglycemia, produced a triphasic response of a rapid onset hypoglycemia, a recovery period from 2-12 hours post treatment and then a prolonged hypoglycemic effect lasting more than 48 hours post-treatment (Kocialski et al., 1972).

An in vitro mechanism of action of the quinoline derivatives, quinolate and 3-mercaptopicolinate, has been studied by Snell (1979). He reported that hepatic gluconeogenesis from lactate or alanine, and the release of alanine from muscle, is inhibited through inhibition of cytosolic and mitochondrial phosphoenolpyruvate carboxykinase. The mechanism involved is a direct effect which is facilitated by complex formation between the agent and Fe$^{2+}$ or Mn $^{2+}$. An inhibitory action on the ferroactivator-mediated Fe$^{2+}$ activation of cytosolic phosphoenolpyruvate carboxykinase, and indirect effects by lowering of cytosolic oxaloacetate concentrations through blocking the translocation of anions such as 2-oxoglutarate from mitochondria, and inhibiting cytosolic aspartate aminotransferase. The active alkaloids of Catharanthus could serve as models for the development of new antidiabetic drugs.

Eleven indolizine alkaloids, synthesized as analogs of vincamine, vindoline, and vindolinine, were tested for oral hypoglycemic activity in fasted rats, but the best was only one third as active as tolbutamide (De and Saha 1975).
Fig. 3 Hypoglycemic alkaloids of *Catharanthus roseus.*
3.2.4 Sulfur Compound from Allium

The hypoglycemic principles of onion (*Allium cepa* L., Liliaceae) and garlic (*A. sativum* L.) are the sulfur-containing compounds, allyl propyl disulfide (18) and diallyl disulfate oxide (allicin, 19). They are active in normal and alloxan-diabetic animals and patients with NIDDM, but not pancreatectomized animals. They are believed to act by competing with insulin, which has a disulfide linkage, for endogenous sulfhydryl-rich insulin-inactivating compounds (Augusti et al., 1974, Oliver-Bever and Zahnd 1979). However, an oral feeding study of garlic bulbs given to normal or streptozotocin-diabetic mice showed reduced hyperphagia and polydypsia but no effect on hyperglycemia or hypoinsulinemia (Swanston-Flatt et al., 1990).

![Chemical structures](image)

Fig. 4 Hypoglycemic sulfur compounds from Allium Spp.

3.2.5 Inorganic Ions from Atriplex

The saltbush (*Atriplex balinus* L., Chenopodiaceae) was investigated for antidiabetic activity in sand rats (*Psammomys obesus*). When these rats were treated with salt bush leaves, they did not develop diabetes but when they were fed with laboratory rat chow or fresh vegetables they develop diabetes. The sand rats have a generic predisposition to diabetes that seems to be prevented by the presence of chromium, manganese, and magnesium salts in the saltbush leaves.

Studies of the leaf ash and chromium *in vitro* showed a potentiation of insulin-stimulated glucose utilization by epididymal fat cells of chromium deficient rats. The mechanism may involve Cr\(^{2+}\) inactivation of an insulin-inactivating enzyme (Aharonson et al., 1969, Oliver-Bever and Zahnd 1979). The hypoglycemic activity of the “glucose-tolerance factor” of brewer’s yeast, *Saccharomyces cerevisiae*, which has been attributed
to trivalent chromium (Cr³⁺), chromium potentiate the action of insulin *in vitro* and *in vivo*. Maximal *in vitro* activity requires mineral complexation, e.g., a chromium-nicotinic acid complex. Clinical trial of patients showed an improved efficiency of insulin (Mertz 1993).

Chronic administration of magnesium salts has also been shown to be beneficial in the treatment of NIDDM. Hypomagnesemia is a common finding in diabetic subjects. Magnesium is a necessary cofactor for many enzymes and is involved in protein synthesis. Treatment with magnesium salts resulted in a net increase in acute insulin response and the rate of glucose disappearance after glucose loading (Paolisso et al., 1989; White and Campbell 1993).

Other minerals may also play a role in diabetes pathogenesis and therapy. The protein tyrosine kinase associated with the insulin receptor has been shown to be Mn²⁺ dependent (Reddy and Kahn 1988). Vanadium is another trace mineral whose salts have insulin-like properties in animal models of insulinopenia or insulin resistance *in vitro* and *in vivo*, due to stimulation of glucose metabolism. Like most dietary trace minerals vanadium is toxic in excess so its therapeutic potential is being investigated carefully (Brichard et al., 1991).

### 3.2.6 Amino Acids from Blighia

Ingestion of unripe akee fruit (*Blighia sapida* Koenig, Sapindaceae) causes the often fatal disorder “vomiting sickness” in Jamaica. The emetic constituents were discovered to be the cyclopropanoid amino acid, hypoglycin A (20) and its γ-L-glutamyl dipeptide, hypoglycin B (21), which are also potent hypoglycemic. They appear to act by inhibiting β-oxidase enzymes, thus blocking oxidation of long-chain fatty acids. Since the fatty acids are no longer available as an energy source, hepatic glycolysis is stimulated to provide an alternate source and the increased utilization of glucose brings about a fall in blood glucose levels. Hypoglycin A is twice as potent a hypoglycemic as hypoglycin B, the latter is also teratogenic, so these compounds are too toxic to be used therapeutically. These compounds may be used as models for the development of new hypoglycemic agents (Feng and Patrick 1958, Von Holt et al., 1966, Tanaka et al., 1972, Oliver-Bever and Zahnd 1979).
In order to find a more specific inhibitor of free fatty acid oxidation, Kanamam et al (1985) screened microbial metabolites for substances that would inhibit the oxidation of long-chain fatty acids in rat liver mitochondria. This research led to the discovery of the β-aminobetaines, emericedin (22) and its more potent synthetic derivative emeriamine (23), from the fungus *Emericella quadrilineata* IFO5859 (Trichocomaceae). Emeriamine has been shown to be a potent and specific inhibitor of carnitine palmitoyltransferase I and both compounds produce dose-dependent oral hypoglycemic and antiketogenic activities in fasted normal, streptozotocin-diabetic, and genetically obese (Zucker) rats.

![Inhibitors of fatty acid oxidations](image)

**3.2.7 Guanidines from Galega**

Seeds of the traditional antidiabetic plant, “goat’s rue,” (*Galega officinalis* L., Fabaceae) contain the guanidine derivative, galegine (24 in Fig 6). Like synthetic biguanide hypoglycemics (25, 26), galegine blocks succinic dehydrogenase and cytochrome oxidase, thus increasing anaerobic glycolysis and decreasing gluconeogenesis. They also cause enhanced glucose uptake and hypoglycemia. Biguanides are also known to inhibit glucose absorption from the intestine (Oliver-Bever and Zahnd 1979).
3.2.8 Vitamins, Coumarins and Steroids from Trigonella

Fenugreek (*Trigonella foenum-graecum* L.), seeds contain a number of hypoglycemic principles. An oral feeding study performed with normal and streptozotocin diabetic mice however did not show significant effect of seed consumption on basal glucose and insulin, insulin-induced hypoglycemia, glycosylated hemoglobin, or pancreatic insulin concentration (Swanston-Flatt et al., 1989). Trigonelline (Fig 1, 6) which is the N-methyl derivatives and main human metabolite of the vitamin nicotinic acid (niacin, 4), has a weak and transient hypoglycemic effect when administered orally to diabetic patients it acts by slowing the metabolism of nicotinic acid, also present in Trigonella, which is known to increase glucose uptake from the blood and its subsequent oxidation. Nicotinic acid is hyperglycemic if administered parenterally, by means of impairment of carbohydrate utilization (Mishkinsky et al., 1967, Shani et al., 1974). Taken orally, nicotinic acid is converted in the body into nicotinamide, which is an inhibitor of the enzyme poly (ADP-ribose) synthetase and is responsible for the depletion of NAD from pancreatic β-cells. It is also a potent hydroxyl-radical scavenger, by which it can prevent the β-cell toxicity of streptozotocin and alloxan (Ledoux et al., 1988). Free-oxygen radicals are important mediators of β-cell destruction in IDDM, and
nicotinamide's antioxidant activity has been shown to have some effect on preventing IDDM and has a slight effect on residual insulin secretion in newly diagnosed patients. Other antioxidants have been tested in animal models with results suggesting prevention of diabetes (Ludvigsson 1993).

Vitamin E (α-tocopherol, 27 in Fig 7), which occurs in seed oils and green leafy vegetables, has been shown at doses of 600-1200 mg daily to reduce the levels of glycosylated hemoglobin in diabetic subjects independently of changes in plasma glucose, which may help reduce the incidence of diabetic complications (Ozden et al., 1989; Ceriello 1991).

Coumarin (28), another constituent of Trigonella, is hypoglycemic in normal and alloxan-diabetic rats (Shani et al., 1974). The mechanism for this observation probably involves hepatotoxicity. Coumarin is hepatotoxic in rats and dogs, where it is metabolized through 3-hydroxycoumarin to reactive quinone metabolites that bind covalently to microsomal proteins. In humans and other primates, however, coumarin is metabolized through 7-hydroxycoumarin to a glucuronide conjugate that is rapidly excreted, and no hepatotoxicity occurs (Cohen 1979). Scopoletin (29), another coumarin constituent of Trigonella, exerts borderline hypoglycemic effects in normal and alloxan-diabetic rats at high doses (Shani et al., 1974). Fenugreekine (30), a steroidal sapogenin-peptide ester, is another hypoglycemic constituent (Ghosal et al., 1974a).
3.2.9 Complex Carbohydrates and Postprandial Blood Glucose

Seeds of a number of other members of fabaceae are used traditionally to treat diabetes. In addition to direct hypoglycemic effects of their constituents, dietary effects are also important. Clinical studies of high legume diets showed improvement in many of the indices of blood glucose control, especially postprandial levels. Beans contain high complex carbohydrates which are more slowly digested than other types of starch. Non-cellulosic types of dietary fiber such as carob gum and guar gum, high-molecular weight galactomannans from *Ceratnia siliqua* L. and *Cyamopsis tetragonoloba* (L.) Taub., respectively, slow intestinal absorption of glucose by slowing gastric emptying and by thickening the unstirred water layer adjacent to the intestinal villi (Leeds 1981, Karlstrom et al., 1987). Modification of the physical and chemical characteristic of the intestinal contents by leguminous gums might also modify the release of gastrointestinal motility (Forestieri et al., 1989). Provision of purified guar fiber as tablets taken with meals significantly reduced low-density lipoprotein cholesterol levels but did not improve excessive postprandial glycemia in NIDDM patients in whom near-normal fasting plasma glucose levels had been obtained with diet, sulphonylureas, or human ultralensatre insulin therapy (Holman et al., 1987). Patient compliance may be a problem with pure guar gum due to its unpalatability and tendency to cause abdominal distension and diarrhea, but
incorporation into high-carbohydrate foods has been shown to provide even more effective blunting of the postprandial glycemic profile without gastric disease (Briani et al., 1987)

Some legumes also contain low levels of lectins, which if incompletely destroyed by inadequate cooking, might accelerate intestinal motility and increase mucus secretion, thus modifying absorption of glucose (Leeds 1981). The antidiabetic activities of a number of other plant gums were attributed to inhibition of gluconeogenesis and stimulation of peripheral glucose utilization, but not to interference with intestinal absorption of glucose (Al-Awadi and Guma 1987). Some structure-activity relationships of hypoglycemic plant mucilage have been studied (Tomoda et al., 1987). Intestinal bacterial fermentation of leguminous oligosaccharides and fiber, in addition to producing a feeling of satiety that might aid in compliance with a fixed diet. It also produces short-chain fatty acids which are then absorbed and affect metabolic processes relevant to diabetic control, such as hepatic gluconeogenesis (Leeds 1981).

A microbial product, acarbose (31 in Fig. 8) isolated from strains of *Actinoplanes* s. (in the order Actinomeces) (Hillebrand 1987), is known to inhibit the intestinal α-glucosidases, λ-amylase, sucrase and maltase. This action reduces the release of glucose from carbohydrates, resulting in a dose-related delay in, or reduction of, the postprandial increase in blood glucose and triglycerides, diminished prevalence of diabetic nephropathy, as well as increased insulin binding in muscle (Hillebrand 1987, Yoshikuni 1988, Le Marchand-Brustel et al., 1990, Hanefield et al., 1991).

Castanospermine (32), an indolizidine alkaloid isolated from *Castanospermum australe* A. Cunn. (Fabaceae), is another example of an intestinal enzyme inhibitor with hypoglycemic activity. Structurally, castanospermine shares similarities with pyranose form of glucose in orientation of its hydroxyl group. It blocks the hyperglycemic response to oral doses of sucrose through inhibition of disaccharase, but does not reduce glucose-induced hyperglycemia (Rhinehart et al., 1987). Moranoline (33), isolated from mulbers (*Morus alba* L., Moraceae) root bark and also leaves of *Jacobinia* (Acanthaceae) and cultures of Bacillus and Streptomyces, inhibits intestinal α-glucosidase potentially but only weakly inhibits β-glucosidase, glucoamylase and α-amylase (Yoshikuni 1988).
Miglitol (34), prepared semisynthetically from moranoline, is an α-glucosidase inhibitor which, unlike acrbose, is absorbable from the gastrointestinal tract. It may exert inhibitory effects on nonsteroidal α-glucosidase present in various cell types, and has been clinically evaluated as a hypoglycemic agent in both IDDM and NIDDM (Reuser and Wisselaar 1994).

Fig. 8 Hypoglycemic intestinal enzyme inhibitors.

3.2.10 Hypoglycemic Glycans

Hikino's research group (Hikino et al., 1985a-c; 1986a-c, 1988; Konno et al., 1985a; Takahashi et al., 1985 a,b, 1986; Tomoda et al., 1987, 1990) has isolated a variety of glucans, peptidoglycans and heteroglycans from plants used in oriental traditional
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medicine. These complex carbohydrates, with molecular weights ranging from approximately 1000 to 10,000,000 amu, were shown to have remarkable hypoglycemic activity when administered intraperitoneally (i.p.) to normal, alloxan-hypoglycemic, and spontaneous diabetic mice.

The mechanism of action of the glucan aconitan A, from Aconitum Carmichaeli Debeaux (Ranunculaceae), involves significant potentiation of the activity of hepatic phosphofructokinase. Acceleration of glycolysis in the liver was accompanied by some increase in hepatic total glycogen synthetase, but liver glycogen content and plasma and liver cholesterol and triglyceride contents were unchanged, indicating that the conversion of glucose into glycogen or lipids does not contribute to the hypoglycemic activity of aconitan A. Plasma insulin levels and insulin binding to isolated adipocytes also were unaffected. Stimulation of glucose uptake and metabolism in small intestine tissues was observed. Thus, stimulation of glucose utilization in the liver and peripheral tissues is the main mechanism for the hypoglycemic activity of aconitan A (Hikino et al., 1989a).

Ganoderm B, a glycan from Ganoderma lucidum Karsten (polyporaceae), increases the plasma insulin levels in normal and glucose-loaded mice, increases the activities of hepatic glucokinase, phosphofructokinase, and glucose-6-phosphate dehydrogenase, decreases the activities of hepatic glucose 6-phosphate dehydrogenase, and reduces hepatic glycogen content. The observed stimulation of glucose metabolism in a homogenate of the small intestine suggests that acceleration of glucose utilization may also occur in peripheral tissues (Hikino et al., 1989b).

Panaxans A-E, glycans of ginseng (Panax ginseng C.A. Meyer, Araliaceae), show different mechanisms of action despite their similar structures. Panaxans A and B stimulate hepatic glucose utilization by increasing the activity of glucose-6-phosphate dehydrogenase, phosphorylase-a, phosphofructokinase. Panaxan A decreases the activity of glucose-6-phosphatase but does not affect hepatic glycogen content. Panaxan B has no effect on glucose-6-phosphatase but decreases glycogen synthetase activity and hepatic glycogen content. Panaxan A does not affect plasma insulin levels and insulin sensitivity, but Panaxan B elevates the plasma insulin levels by potentiating insulin secretion from
pancreatic islets and enhances insulin sensitivity by increasing insulin binding to receptors (Suzuki et al., 1989a, b).

Ginseng contains a number of other hypoglycemic constituents, with different mechanism of action. Adenosine was isolated from a water extract of the rhizomes by bioassay guided fractionation, and was shown to enhance lipogenesis and cyclic adenosine monophosphate (cAMP) accumulation in adipocytes, which possess specific adenosine receptors. Some of the sterol glycosides known as ginsenosides (35 in Fig. 9) inhibited adrenocorticotropin-induced lipolysis and at same doses suppressed insulin-stimulated lipogenesis, while others stimulated the release of insulin from cultured islets (Waki et al., 1982; Ng and Yeung 1985).

Fig. 9 Sapogenin of ginsenosides and panaxosides; protopanaxadiol \( R_3=H \), protopanaxatriol \( R_3= \text{OH} \); sugars in glycosides are attached to oxygens at \( R_1=R_3 \)

3.2.11 Plant Constituent that Modulate Intracellular Second Messengers

Pancreatic \( \beta \)-cell membranes possess adenosine triphosphate (ATP)-sensitive \( K^+ \) channels which, in the absence of glucose, allow an efflux of \( K^+ \) to contribute a hyperpolarizing membrane current that maintains the hyperpolarized resting membrane potential of the cell. Metabolites of glucose and amino acids inhibit this channel, causing a reduction in the hyperpolarizing current, which leads to \( \beta \)-cell depolarization and voltage dependent \( Ca^{2+} \) uptake. Binding of \( Ca^{2+} \) to calmodulin results in the microfilament contraction, resulting in exocytosis of insulin from storage granules. Intracellular ATP is believed to have a second messenger role in inhibiting the \( K^+ \) channel by almost 99%, thus making the \( \beta \)-cell very sensitive to changes in channel activity (Cook et al., 1988, Misler et al., 1989). Tolbutamide specifically mimics the effects of
glucose stimulation, depolarizing the β-cells by inhibiting the ATP-sensitive K⁺ channel, which has been suggested to be the β-cell receptor for sulphonylureas. The alkaloid quinine (36) is also a potent blocker of this channel, although, unlike the sulphonylureas, it also blocks Ca⁺²-activated K⁺ channels (Cook and Ikeuchi 1989).

Intracellular cAMP also acts as a second messenger in the β-cell. Increasing the intracellular cAMP concentration potentiates cholecystokinin and glucose stimulated insulin release. The mechanism involves synergistic action with the influx of Ca⁺² that occurs as a consequence of the glucose metabolite induced increase in intracellular K⁺ (Hill et al., 1987). The physiological actions of glucagons result from stimulation of cAMP synthesis, which in pancreatic β-cells forms part of the pancreatic hormone regulatory mechanism (Larner 1980). The role of second messengers in insulin action has been reviewed by Saltiel (1990).

The most famous plant product for the stimulation of intracellular cAMP is forskolin (37), a diterpene from Coleus Forskohlii (Poir.) Briquet (Lamiaceae). It is an adenylate cyclase activator which increases intracellular cAMP by stimulating its biosynthesis. Theophylline (38) and other methylxanthenes from Camellia sinensis (L.) Kuntze (Theaceae) and Ilex guayusa Loesner (Aquifoliaceae), and papaverine (39) from Papaver somniferum L. (Papaveraceae), are phosphodiesterase inhibitors which increase intracellular cAMP by preventing its breakdown (Gearien and Mede 1981, Hill et al., 1987, Zawalich et al., 1988). Theophylline is orally hypoglycemic when administered chronically to normal rats, but this in vivo effect was not attributed to its phosphodiesterase inhibition, but rather due to its intracellular Ca⁺² efflux. Increased extracellular Ca⁺² might enhance calcium stimulated ATPases, which would result in decreased cellular ATP levels, enhanced lipolysis and reduced glycogenolysis. This effect is also seen when caffeine is administered (40) (Tobin et al., 1976).

Sodium salicylate (salt of 7) inhibits cyclooxygenase, thus preventing the metabolic cascade from arachidonic acid to the prostaglandins. Inhibition of β-cell PGE₂ synthesis increases glucose-induced insulin secretion because this prostaglandin binds to specific β-cell receptors that are coupled to regulatory components that inhibit adenylate cyclase. Inhibition of this enzyme would lead to decrease in intracellular cAMP
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(Robertson 1988). Additionally, arachidinic acid (41) itself is an insulin secretogogue, acting to mobilize Ca^{2+}, increasing its free cytosolic concentration, and to activate protein kinase C (Metz 1988).

Carbohydrate components of the diet stimulate the release of the hormone "gastric inhibitory polypeptide," which is thought to influence insulin secretion by elevating islet of β-cell cAMP levels. The activity of cAMP is also synergized by phosphoinositidinederived second-messenger molecules generated during the phospholipase C-mediated cleavage of membrane phospholipids in the β-cell. This hydrolysis is thought to be activated by the interaction of extracellular hormones and agonists with a specific membrane receptor (Zawalich 1988).

The flavonoid, (-)-epicatechin (42), isolated as the active principle of the traditional antidiabetic plant *Pterocarpus marsupium* Roxb. (Fabaceae), has been shown to cause an ATP-dependent enhancement of glucose-stimulated insulin secretion from isolated islets, and to cause a rise in islet insulin content invivo in rats. Inhibition of cAMP phosphodiesterase and stimulation of insulin biosynthesis were suggested to be the mechanism for the observed effects (Hii and Howell 1984). The flavonoids quercetin (43) and myricetin (44) have also been reported to be hypoglycemic (Rahman and Zaman 1989), but they are known to be potent inhibitors of tyrosine kinase (Geahlen et al., 1989), the activity of which is essential in the post-receptor-binding activity of insulin.

When insulin binds to the extracellular α-subunit of its heterodimetic cell surface receptor, the insulin receptor complexes aggregate along the plasma membrane and are then internalized rapidly. Activation of a Mn^{32} -dependent protein tyrosine kinase in the transmembrane β-subunit ensues, resulting in phosphorylation of the receptor and other proteins with phosphate groups from ATP (Reddy and Kahn 1988). Activation of a phosphotidylinositol-specific phospholipase C leads to hydrolysis of a membrane glycan phosphoinositide. This produces a cyclic inositol phosphate-glucosamine second messenger that activates phosphodiesterase, decreasing intracellular cAMP, and also produces diacylglycerol, which activates protein kinase C (Saltiel et al., 1986). Protein kinase C regulates a number of enzymes and the insulin receptor though phosphorylation (van de Werve 1985a).
Some tumour-promoting phorbol esters, such as 12-O-tetradecanoylphorbol-13-acetate (TPA, 45), share structural similarities with diacylglycerol, and are potent activators of protein kinase C (van de Werve et al., 1985). Phorbol esters are diterpenes isolated from species of *Euphorbia* and a few other genera of the Euphorbiaceae (Kinghorn 1983), 30 species of which have been associated with the treatment of diabetes. Phorbol esters have been reported to have a number of insulinomimetic effects, including stimulation of glucose transport, lipogenesis, and amino acid uptake. However, they may reduce insulin receptor affinity for insulin, insulin stimulation of glucose transport and lipogenesis, and basal glycogen synthesis (Sowell et al., 1988). It has been suggested that phorbol ester-stimulated serine phosphorylation of insulin receptors may be associated with a decrease in the affinity of the receptor for insulin and decreased receptor tyrosine kinase activity, although conflicting results have been reported (van de Werve 1985a; Obermaier et al., 1987; Sowell et al., 1988). Ishizuka et al., (1991) found that phorbol esters, glucose and insulin translocatively activate protein kinase C, resulting in a subsequent down regulation of protein kinase C and insulin stimulate glucose uptake in adipocytes. This contributes to impaired responsiveness of the glucose transport system after prolonged insulin and/or glucose exposure. Phorbol esters can inhibit $\alpha_1$-adrenergic stimulation of glucose production by inhibiting phosphorylase activity, also through their effect on protein kinase C (van de Werve 1985a). They can also inhibit glucagons-stimulated adenylate cyclase, but the metabolic significance of this is much less than that of their inhibition of $\alpha_1$-adrenergic effects (Garcia-Sainz et al., 1985). Tumour promotion may also be explained by phorbol ester activation of protein kinase C (van de Werve 1985a).
3.2.12 Plant Hypoglycemic Acting by Adrenergic Effects

In addition to the \( \alpha_1 \)-adrenergic inhibition described above for tumor-promoting phorbol esters, a number of alkaloids are known to affect blood glucose levels by a similar mechanism. In normal patients, there is no effect of \( \alpha_1 \), \( \beta_1 \), or \( \alpha_1 + \beta_1 \)-blockade on the slope of glucose-potentiated insulin secretion. In patients with NIDDM, only selective \( \alpha_1 \)-adrenergic blockade increases glucose-potentiated insulin secretion, through both a decrease in an endogenous overactive \( \alpha_1 \)-adrenergic stimulation and an increase in synaptic cleft norepinephrine levels, which results in an increase in islet \( \beta \)-adrenergic stimulation. Thus, a chronic decrease in islet \( \alpha_1 \)-adrenergic stimulation may be a useful adjunct to NIDDM management (Broadstone et al., 1987).

Ergot alkaloids, occurring in fungi such as *Claviceps purpurea* (Fries) Tulasne (hypochnaceae) *Rivea corymbosa* (L.) Hallier f. and closely related *Ipomea* and *Argenia* species (Convolvulaceae) are \( \alpha \)-adrenergic blockers which inhibit epinephrine induced hepatic glycogenolysis and hyperglycemia. These effects are not correlated with their well-known smooth muscle effects, and may not be due to a specific \( \alpha \)-receptor (Weiner 1980). Dihydroergotamine (46 in fig. 11) and yohimbine (47), another \( \alpha \)-adrenergic blocking alkaloid from *Pausinstalstia yohimbe* (K. Schumann) Pierre (Rubiaceae), prevented epinephrine-induced inhibition of insulin release, but not diazoxide-induced
inhibition (Henquin et al., 1982). Yohimbine is also a monoamine oxidase inhibitor and is contraindicated for patients with diabetes.

Beta-adrenergic blocking agents reduce the hyperglycemic response to epinephrine by blocking its stimulation of cAMP production. Epinephrine-induced glycogenolysis in heart and skeletal muscle and lipolysis in isolated rat adipocytes is inhibited. By these mechanisms, the non-selective β-adrenergic blocking agent, propranolol, slows the post-insulin recovery of glucose concentration and prevents the usual rebound of plasma glycerol, while not affecting plasma glucose or insulin concentrations in normal individuals, or the rate or magnitude of the fall of plasma glucose after insulin (Weiner 1980). Beta-adrenergic blocking agents can also reduce insulin resistance caused by β-adrenergic simulation (Attvall et al., 1987). Kimura et al., (1988) suggested a possible β-adrenergic blockade mechanism for the hypoglycemic activity of an orally-administered aqueous extract of *Ganoderma lucidum* (Leyss. ex Fr.) P. Karst (Ganodermataceae).

Reserpine (48) from *Rauvolfia serpentina* (L.) Benth. Ex Kurz (apocynaceae), is an adrenergic blocking agent that causes intracellular depletion of catecholamines and serotonin. Uptake of catecholamines is also antagonized by inhibition of the ATP-Mg$^{2+}$-dependent uptake mechanism of the chromaffin granule membrane. A transient sympathomimetic effect is seen only after parental administration of relatively large doses; pharmacological effects of the released catecholamines are minimal unless monoamine oxidase has been inhibited (Weiner 1980). Reserpine enhanced the hypoglycemic effect of insulin and the hyperglycemic effect of insulin and the hyperglycemic effect of epinephrine in normal subjects. In glucose tolerance tests it inhibited the hyperglycemic response, even in diabetic patients (Ricci and Ricordati 1955). However, hyperglycemia is not reported as a significant side-effect of reserpine, nor is interactions with other hypoglycemic drugs listed (American Pharmaceutical Association 1976).
3.2.13 Photosensitizers and IDDM

Insulin dependent diabetes may arise through T-lymphocyte mediated β-cell destruction. One possible novel approach to interrupting this pathogenic process is photopheresis, whereby lymphocytes would be treated with a photosensitizer such as 8-methoxypsoralen (49 in Fig. 12) and UV radiation to cause a change in the antigencity of the treated lymphocytes. This is postulated to cause a vaccination-like effect in the patient when they are retransfused at repeated intervals into the patient. This has proved effective in other autoimmune diseases and is now in clinical trials for IDDM (Ludvigsson 1993). Photosensitizers have been isolated from more than 30 flowering plant families (both}
monocots and dicots) and represent a wide range of chemical classes including: polyacetylenes, thiophenes, lignans, porphyrins, quinines, chromenes, benzofurans. Furoflavonoids, furocoumarins (e.g. 49), furochromones, furoquinoline alkaloids, and β-carboline alkaloids (Downum 1986, Hudson 1990). A thiophene such as α-terthienyl (50) may have an advantage over 8-methoxypsorelan in these applications because of its lack of genotoxicity (MacRae et al., 1980, Tuveson et al., 1986). Structure-activity relationship studies of thiophenes have shown the possibility of achieving some cell or organism specificity despite the general mechanism of action involving singlet oxygen generation (Marles et al., 1992).

Fig. 12 Plant derived photo sensitizers
Table 5. Chemical constituents and its mechanism of action.

<table>
<thead>
<tr>
<th>Class</th>
<th>Plants</th>
<th>Constituents</th>
<th>Mechanism</th>
</tr>
</thead>
<tbody>
<tr>
<td>Indole</td>
<td></td>
<td>Indole-3-acetic acid, Indole-3-propionic acid</td>
<td>Inhibit insulinase</td>
</tr>
<tr>
<td>Alkaloids</td>
<td><em>Catharanthus roseus</em></td>
<td>Catharanthine, leurosine, Lochnerine, Vindoline, Tetrahydroalstonine, Vindolinine</td>
<td>Increase ATP content, Increase in lactate/pyruvate ratio</td>
</tr>
<tr>
<td>Steroid glycosides</td>
<td><em>Momordica chirantia</em>, <em>Momordica foetida</em></td>
<td>β-sitosterol-D-glucoside, 5-25 stigmastadein-3-β-ol-D-glucoside</td>
<td>Inhibition of glucose uptake</td>
</tr>
<tr>
<td>Quinidine</td>
<td></td>
<td>Quinolate, 3-mercaptopicolinate</td>
<td>Hepatic gluconeogenesis from lactate or alanine</td>
</tr>
<tr>
<td>Sulfur containing compound</td>
<td><em>Allium cepa, Allium sativum</em></td>
<td>Allylpropyl disulphide, Diallyldisulfate oxide</td>
<td>Increase insulin secretion</td>
</tr>
<tr>
<td>Amino acids</td>
<td><em>Blighia sapida</em></td>
<td>Hypoglycin A, Hypoglycin B</td>
<td>Inhibit β-oxidase enzymes, Blocks oxidation of long chain fatty acid, Increase utilization of glucose</td>
</tr>
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<td>Guanidine</td>
<td><em>Galega officinalis</em></td>
<td>Galegine</td>
<td>Succinic dehydrogenase and cytochrome oxidase inhibitors</td>
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<tr>
<td>Vitamins</td>
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<td>Nicotinic acid</td>
<td>Antioxidant</td>
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<td>Coumarin</td>
<td><em>Trigonella foemum-graecum</em></td>
<td>Trigonella, Scopoletin</td>
<td>Hepatotoxicity</td>
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</table>

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<table>
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<th>Class</th>
<th>Constituents</th>
<th>Plants</th>
<th>Mechanism</th>
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<td>Complex carbohydrate</td>
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<td>Ceratonia siliqua, <strong>Cyamposis tetragonoloba</strong></td>
<td>Inhibition of intestinal glucose absorption</td>
</tr>
<tr>
<td>Indolizidine alkaloid</td>
<td>Castanospermine, Moronoline</td>
<td>Acosmum Carmichaeli, <strong>Ganoderma lucidum</strong>, Panax ginseng</td>
<td>Inhibition of α-glucosidase, Potentiation hepatic phosphofructokinase, Glucose-6-phosphate dehydrogenase</td>
</tr>
<tr>
<td>Glycans</td>
<td>Forskolin</td>
<td><strong>Coleus forskohii</strong>, Pterocarpus morsypium</td>
<td>Increase intracellular cAMP, Inhibit tyrosine kinase, ATP dependent enhancement of glucose stimulated insulin secretion</td>
</tr>
<tr>
<td>Flavanoids</td>
<td>Peptides</td>
<td><strong>Monarda chirantia</strong>, Euphorbia Species</td>
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<td>Phorbol esters</td>
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3.3 Introduction to standardization of herbal materials

Various systems of plant based traditional medicine have been in use in India and also in many parts of the world for ages. Before the advent of modern medicine, the traditional systems of medicine were playing a central role in healthcare. According to an estimate, majority of the world population, especially in the developing countries, still depends on herbal products for their primary health care needs, possibly for the following reasons: 1) lack of easy access to drugs of modern systems, 2) popular belief that herbal drugs are free of adverse effects, 3) low price of herbal drugs as against prohibitive cost of most of the allopathic drugs, 4) concern over the toxicity and side effects of modern drugs. The traditional medicine has been steadily gaining interest and acceptance even amongst the practitioners of modern medicine (Rajani and Padli 2000).

Plants have also been a source of chemical substances which serve as drugs in their own right or as key ingredients in synthetic drugs. According to an estimate, 42% of the prescriptions dispensed in US and 50% in Europe had at least one active ingredient of plant origin. Natural products have been playing a pivotal role in the discovery of new pharmaceuticals as well. One of the latest examples is the discovery of taxol, a diterpene isolated from the stem bark of Taxus brevifolia, the most significant anticancer agent developed in the last two decades. The other examples include vincristine, vinblastine, etoposide, teniposide and artemisinin.

World health organisation currently encourages, recommends and promotes traditional/ herbal remedies in national healthcare programs in developing countries mainly to reduce financial burden on the respective governments. Plant materials and herbal remedies derived from them represent a substantial proportion of global drug market and in this respect internationally recognized guidelines for their quality assessment are necessary.
3.3.1 Need for Standardization

In Indian systems of medicine there are about 1000 single drugs and 1500 formulations. These drugs are fully documented in traditional texts for their therapeutic properties. However, the difficulties in identification of appropriate plant species, their geographical source, time of collection, drying and storage conditions, microbial contamination and presence of xenobiotics, amongst other factors, result in extensive and unpredictable variation in the quality of the raw material, which reflects eventually in the formulations as well. Hence, to ensure quality and efficacy, there is a need to develop methods for standardization of raw material. Moreover, for pharmaceutical purpose, the quality of the medicinal plant material must be as high as that of other medicinal preparations.

Most of the herbal formulations, especially the classical formulations of traditional medicine, are polyherbal. Each formulation contains 10-20 ingredients, a few have been 50-75. Many preparations are either liquids or semisolid. For such formulations it is very difficult to establish parameters for quality control. Moreover, the unique processing methods followed for the manufacture of these drugs turn single drugs into very complex mixture, from which separation and identification, leave alone the analysis of the components, is a Herculean task.

3.3.2 Problems in Standardizations

Problems in standardization arise from the complex chemical composition of herbal drugs. Standardization of certain marker compounds of a herbal drug in general does not serve the purpose of standardization since activity of the drug does not depend upon one or a few chemical components. In most of cases, it is the result of concerted activity of several active compounds as well as of inert accompanying substances. Though these inert components do not directly affect the activity of the drug, they might influence bioavailability and excretion of the active of the active components. Further, these inert chemical components may also play a role in the stability of the active component and minimize the rate of side effects. If there are several active compounds present in a herbal drug, they may have additive or potentiating effect. The quantity of the active constituents in the drug may be influenced by several factors such as age and
origin, harvesting period and so on. To eliminate at least some of the causes of inconsistency, in terms of active ingredients, it was suggested that one should be cultivated plants rather than wild plants which are often heterogeneous with respect to the above factors and consequently in their content of active principles. (Handa 1995).

3.3.3 Quality Control Parameters for Herbal Raw Material

Consistent quality of herbal medicinal products can only be assured if the starting materials are defined in an explicit and rigorous manner. A general protocol followed for quality control of raw material is shown in Figure 13 (Handa, 1995).

3.3.3.1 Authentication

The plant material should be collected from various geographical sources at appropriate stage of growth to ensure the quality. Authentication is carried out by detailed taxonomical studies so that chances of deliberate or unintentional adulteration or substitution are avoided.

3.3.3.2 Foreign matter

Plant parts other than those constituting the drug are considered as foreign matter. This also includes any other matter of plant or mineral origin present in the drug sample. The medicinal plant material should be entirely free from soil, stones, dust, insects and other animal contamination including animal excreta.

3.3.3.3 Organoleptic evaluation

Organoleptic evaluation refers to evaluation of the material by means of sense organs and includes the macroscopic appearance of the drug including its form, surface and size, odour, taste, feel to the touch. It is advisable to compare the drug sample with reference drug to check variability due to individual human perception. The colour of the drug is inspected in diffuse daylight and should match, or be close to the reference sample. When colour is described in a combination of two colours the latter is the main colour. The odour and the taste should only be determined if the drug is known to be non-toxic. Aromatic drugs should be gently crushed to observe the odour.
3.3.3.4 Microscopic examination

Microscopic examination is one of the important tools for the identification of the plant and also for the study of adulterants. By this technique specific character of the tissue, cells and cell content, powder characteristics of the drug can be identified. Diagnostic microscopic features like type of stomata, trichomes, fibres, vessel thickenings and cell content are very important. Quantitative microscopy of drug, which contain content is specified in their official monographs.

3.3.3.5 Ash value

Contamination a constant number of some parameters like stomatal number, stomatal index and palisade ratio, is of immense value in identification of closely related species.

3.3.3.6 Volatile matter

Volatile oil content of the drug is determined by water distillation using standardized apparatus designed for this purpose. For various aromatic drugs volatile oil of the plant material with earthy material can be identified by incineration of plant drugs, which will leave organic ash. The percentage of ash like total ash, acid-insoluble ash, water soluble ash and sulphated ash are determined using standard procedure described in official documents. It will serve as an indicator of care taken during the processing of plant material, especially for underground parts.

3.3.3.7 Extractive values

The determination of extractable matter refers to the percentage of matter extracted from the drug using specified quantity of solvent. Extractive value provide indication of presence of polar, non-polar and medium polarity components present in the plant material. It serves as a quick indicator of quality of the pant material.

3.3.3.8 Pesticide residues

Various biocidal agricultural chemicals, collectively known as pesticides, which are widely used to reduce the presence of insects, fungi and moulds in food. However their excessive and irrational use has resulted in contamination of soil and water. The
toxic residue in medicinal plant material results from soil and water because these pesticides are used during cultivation of medicinal plants or fumigation during storage. Since many herbal preparations are taken over long period of time, limits for pesticide residue should be established by using modern instruments. Special emphasis is paid to checking the persistent organic pollutants like DDT, aldrin, dieldrin and toxaphene congeners which are not allowed in medicinal plants.

3.3.3.9 Heavy metal contamination

Contamination of medicinal plant products with metals like arsenic, lead, cadmium, mercury and nickel can attributed to many causes especially to environment pollution from industrial activity. The limits in parts per million of such heavy metals in medicinal plants should remain within specification.

3.3.3.10 Microbial contamination

Medicinal plant material harvesting, handling and production often cause additional contamination and microbial growth. Large number of bacteria and fungi forms the naturally occurring microflora of the herb. There is a need to specify the total count of aerobic microorganism, yeast, moulds and the absence of specially objectionable microorganisms. Determination of Escherchia coli and mould may reflect the care taken during production or harvesting. Microbial count should be determined according to the pharmacopoeial or other validated procedure.

3.3.3.11 Radioactive contamination

After plant material harvesting, irradiation may have been used as procedure for microbial decontamination and sterilization. As well as effluent from adjoining companies may contain radioactive contaminants and flow into an area where plant material is collected. Under all such circumstances strict WHO guidelines should be followed.
3.3.4 Assay for Active Constituents/Marker

The quality of drug depends on the content of active constituents, the amount of which depends up on various factors that affect the quality of crude drug. Where ever possible if the active constituent is known with certainty, it should be analyzed to assure the quality. However in many cases, information regarding active constituent is incomplete or active constituent is unknown. Under these circumstances, any one of the chemically characterized components of plant material called chemical marker can be used as a reference for evaluating the quality of the plant material. Thus the marker is a constituent of a medicinal plant material that is chemically defined and of interest for quality control purpose. Most appropriate is a biomarker i.e. the active compound which is responsible for biological activity. When only inert chemical constituent are known from plant, a judicious selection of one of them for marker purpose should be made giving priority to a component specific to the plant under consideration and its stability.

At present, a wide variety of analytical techniques are available for quantification of various components of crude drugs. Chromatographic methods with wide range of sophistication are more important for phytochemical evaluation the crude drugs. Of the many available chromatographic methods available TLC has become widely accepted for rapid analysis of plant drugs. It can be used for qualitative as well as quantitative estimation of components of crude drugs. Qualitative determination can be carried out by fingerprint profiling of the extract. Quantitative estimation is carried out by TLC densitometry. TLC densitometry carries advantage over high performance liquid chromatography as it accepts comparatively unpurified samples without much compromise on the efficiency. More recently, a combination of chromatographic and spectroscopic method has become more popular for drug analysis.

3.3.5 Standardization of Finished Products

After the standardization of raw materials, next step is to monitor the process preparation of the formulation and set parameters for in-house quality control testing and finally the standardization of final product. Quality assurances of herbal products rely upon good manufacturing practices with adequate batch analysis and standard methods of preparation. Various processes used in the manufacture lack standardized methods. Large
scale commercialization of herbal drugs necessitates scientifically evolved standardized methods of plant drug production. General protocols for standardized production of herbal formulation are given in Figure 13 (Handa 1995).

3.3.6 Current Status of Standardization

World health organization in a number of resolutions emphasized the need to ensure the quality of herbs and herbal formulations by using modern techniques. Internationally and in our country too, several pharmacopoeias have provided monographs defining quality parameters and standards for many herbs and herbal products.


In most of these monographs along with identification tests like physico-chemical and microscopical characterization, there is also provision for gravimetric and titrimetric tests for a number of herbs. In some of pharmacopoeias, TLC fingerprint for a number of herbs is recommended (Dobriyal and Narayana 1998) but they lack in tests based on modern analytical techniques like chromatography, spectroscopy etc., and also marker compound analysis.

To ensure quality and efficacy of herbal drugs, phytochemical standardization, using sophisticated analytical techniques is very essential. Phytochemical standardization can be done at two levels:

1. Standardization by chemical/biomarker compound analysis
2. Standardization by fingerprint techniques/chemoprofiling

These are described in the following sections.
Every herb has a range of chemical constituents which are produced as a result of metabolic activities in the plants. These compounds either alone or in combination are mainly responsible for the pharmacological activities or therapeutic action in the human body. Hence it would be more practical to test for the presence of these compounds. For example, Aswangantha (*Withania somnifera*) can be assayed for withanolides, Guggulu (*Commiphora mukul*) for guggulsterones, Neem (*Azadirachta indica*) for azadirachtin or nimbidine, Harida (*Curcuma longa*) for curcuminoids. On the other hand, where the chemical composition of the herbs has been worked out but it is not clearly established whether these chemical entities are responsible for some particular action, any compound which is predominantly present in the herbs can be utilized as marker compound for the purpose of standardization. This group represents compounds like aegelin in bilva (*Aegle marmelos*), shatawarine in shatawari (*Asparagus racemosus*), fistulin in Aravadha (*Cassia fistula*) (Dobriyal and Narayana 1998).

One of the best methods of standardizing herbs and herbal formulations based on the modern scientific tools is using chromatography. It helps not only in establishing the correct identity but also in regulating the chemical sanctity of the herbs. For quantitative work, HPLC is preferred generally. GC is used mainly for volatile components like essential oils and perfumes. In the past few years, HPTLC has emerged as a potential tool for rapid and efficient analysis of extracts of herbal drugs and formulations (Indian Herbal Pharmacopoeia 1998; Rajani et al., 2000; Shah et al., 2000).

### 3.3.7 Standardization of Herbs Through Fingerprinting/Chemoprofiling

The process of chemoprofiling fingerprint technique essentially involves the collection of plant material from different geographical locations, preparing different extracts (by sequentially extracting the plant material with different solvents basing on their polarity) and establishing the chemical pattern of components present in the extract by separating them using chromatographic techniques like HPLC and HPTLC. The pattern thus obtained from the above process is unique for a particular plant (Bhutani 2000).

In the past few years, HPTLC is emerging as a powerful tool for the establishment of TLC fingerprint profile. The parameters to be considered to establish a complete TLC
fingerprint profiles includes distinctive pattern of chromatogram, the migration distances of the compounds separated (Rf), the bands as observed with naked eye, as examined under UV (254 and 366nm), the UV absorption spectra of the resolved compounds., densitometric/ fluorimetric measurements of the resolved compounds for the calculation of their relative percentages and finally response to several reagents during derivatisation.

The main advantage of the fingerprint techniques is that the herbal drugs can be authenticated especially when the active principles are not known or when chemical markers are not available for the analysis. In the absence of known chemical markers the distinctive TLC fingerprint profile would form a benchmark for the drug and can be used to ascertain the quality of the herbal drug. Moreover, in those cases where chemical markers/biomarkers are known and method of analysis established for those compounds, it is still advisable and essential to develop fingerprint profiles to further characterize the herbal drug, since it is believed and in certain cases established that many compounds other that the marker compounds present in the herb have a role in the final therapeutic activity of the drug.
Analysis at the time of use

Inprocess analysis

Analysis

Source: Identification

Proper storage Conditions

Well defined process

Proper storage before packing

Figure 13
3.4. Phytopharmacology of *Enicostemma littorale*

*Enicostemma littorale* Blume is a glabrous perennial herb belonging to the family Gentianaceae. The plant grows throughout India in the hills of up to 1,500 ft. altitude as well as near the sea. It is also found in Malaysia, Java, Celon, tropical South Africa and West Indies (Kirtikar and Basu 1935; Chopra et al.; 1956, Maheshwari 1963). In India the plant is known by different local names in different languages like, Nagajivha in Eastern India (Bengal), Mamejwa in Gujarati, Kadavinayi, Manucha, Memijwa, Naichapida, Tanavadi in Marathi, Chota-kirayat, Chota-chiretta, Chota-chirayata. In Hindi, Vellaragu, Vallari in Malayalam, Krimihrita, Ksharakarma, Kshitaukshupa, Mabhijaka, Magajihva, Manejaka, Mamejeka, Nagajivha, Nahu, Tiktapatra in Sanskrit, Manucha in Sindi, Nelaguli, Nelagulimidi in Telugu.
3.4.1 Morphology of the Plant

It is a glabrous perennial herb growing about 5-15 cm in height, branching from the base. The stem is erect or procumbent glabrous and yellowish green in colour and possesses four bulges which appear as wings at the four corners. The internodes are short. The stem bears numerous leaves and small flowers all around (Kirtikar and Basu, 1935; Chopra et al., 1956; Nadakarni 1954).

The leaves are simple, sessile, opposite and variable, 3.2-6.3 cm by 3-16 mm in size. They are obtuse or acute, linear, elliptic, lanceolate, glabrous, 3-nerved, the midnerv strong and the marginal nerves often obscure. The upper surface is glaucous (Nadakarni 1954; Wealth of India 1952; Gamble 1957).

The flowers are small, white and sessile, in axillary whorled clusters all along the stem. The calyx is 3 mm long, lobes are 1.5 mm long, ovate-oblong, obtuse with narrow membranous margin. The corolla is white in colour and 6-8 mm long and tubular. Its lobes are 2.5 mm long, lanceolate and acute. The stigma is large and bilobed. Stamens are attached to the corolla tube and epipetalous (Kirtikar and Basu 1935; Chopra et al., 1956).

Capsule is 4 mm long, ellipsoid, slightly narrowed at the base, rounded at the apex, apiculate with the remains of the style and contains numerous seeds. The seeds are small. The root is creeping, filiform and white in colour. All parts of the plant are bitter in taste (Wealth of India 1952; Gamble 1957).

3.4.2 Microscopical Characters of the Plant

3.4.2.1 Stem

The stem possesses 4 bulges at the 4 corners, the length of the bulges being 95-163-177-190 microns. The 2 bulges on each side are more or less parallel to each other. The epidermis is composed of a single layer of cubical cells and is covered externally by a thick and striated cuticle. Epidermis stays enraptured even if secondary growth is present. A few stomata of the cruciferous type are found interspersed in the epidermal cells.
Epidermis is followed by the cortex, composed of 1-2 layers of collenchyma (which also fills the whole inner spaces of the bulges) and 5-8 layers of isodiametric parenchymatous cells. Some of which contain acicular crystals of calcium oxalate.

An endodermis is present but not very distinct. Thin layer of pericycle represented by 1-2 layers of cells. Between the phloem and xylem there is a thin layer of cambium which gives rise to secondary tissue.

Phloem is composed of phloem parenchyma (some cells of which are filled with reddish brown amorphous mass), sieve tube elements, accompanied by companion cells. Xylem is composed of few spiral, scalariform, reticulate and mostly pitted vessels, tracheids and xylem parenchyma. Medullary rays are absent.

Centre of the stem is occupied by pith which consists of more or less rounded, isodiametric, parenchymatous cells, having thin walls and intercellular spaces. As the stem grows older some of the pith cells, particularly in the central region get disintegrated. Small acicular and resette crystals of calcium oxalate and rarely prisms are present in pith cells.

3.4.2.2 Leaf

The leaf is isobilateral with stomata of the cruciferous type on both surfaces of the leaf. Hair is absent on both surfaces of the leaf. A transverse section shows one cell thick epidermis, covered externally with a fairly thick and striated cuticle, developed on both surfaces of the leaf, almost to the same extent. Cuticle is thicker at the margin.

Epidermis is followed by the mesophyll, which is represented by 5-9 layers of irregular cells, containing chloroplasts. Rosettes of calcium oxalate and less commonly, prisms are found in some of the cells of the mesophyll.

In the midrib region, the cells of lower epidermis are smaller with thicker walls as compared to upper epidermal cells. Following the lower epidermis is a 1-2 cells thick layer of collenchymatous cells and 5-7 layers of isodiabetic, parenchyma cells. Central region of midrib is occupied by the vascular tissue in which the phloem surrounds the central xylem strand. The phloem is however, more developed on the dorsal than on the ventral side and consists of sieve tubes, companion cells and phloem parenchyma. The
xylem is composed of spiral, scalariform, reticulate and rarely pitted vessels and xylem parenchyma. A few spiral trachieds are also to be found. The smaller veins have a similar structure as midrib. Stomatal index is 17.25 on the upper surface while it is 18.25 on the lower surface (Phadnis 1988).

3.4.2.3 Root

a. Young root

Transverse section shows a single layer of epidermis composed of somewhat cubical cells, arched outside and a wide zone of cortex, consisting of 4-9 layers of parenchymatous cells with large intercellular spaces. Cortex is followed by a distinct endodermis composed of tangentially elongated and somewhat thick walled cells. In some cases possesses a secondary radial cell.

Primary xylem exhibits a tri to heptarch condition, tetrarch and hexarch being more common. Protoxylem strand is represented by just 1 or 2 annular and spiral vessels, which are usually separated from the central metaxylem plate by a few layers of parenchymatous cells. These protoxylem elements remain isolated throughout the growth of the root and are never crushed or embedded by secondary xylem tissue even though the secondary growth may be quite advanced.

b. Mature root

No periderm layer is to be seen but the tissues outside the endodermis get slightly ruptured so that even when secondary growth is fairly advanced the endodermis and pericycle layers remain quite intact. These layers are further characterized by the possession of 1-3 secondary radial walls.

3.4.3 Phytochemical Investigations

Dymock et al., (1893) reported that aerial part of the plant gave 34 % of dry alcoholic extract and 15.7 % of ash, while the subterranean part gave 15.5 % of dry alcoholic extract and 10.4 % of ash. Water soluble ash of the whole plant was found to be 2.08 % while acid insoluble ash content was found to be 15.7 %. Qualitative analysis of the ash revealed the presence of iron, potassium, sodium, calcium, magnesium, silica, phosphate, chloride, sulphate and carbonate. The presence of n-hexacosanal, heptacosane
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and nonacosane in the alcohol-insoluble portion of unsaponifiable matter and myristic, stearic and oleic acids in the saponifiable matter of the petroleum ether extract have been reported by (Mehta and Devani 1959).

Prasad and Bhusan (1954) isolated an alkaloid with m.p. 79-80 °C and phytosterol in their preliminary investigation of the plant. Sharma and Jain (1960) isolated two alkaloids; one was greyish white in colour, optically inactive with m.p. of 80-82 °C and molecular weight of 180. The second alkaloid was in the form of brown scales, showed shrinking at 45 °C and melted completely between 58-60 °C. Natarajan and Prasad (1972) isolated four chloroform soluble alkaloids, one water soluble alkaloid, two sterols and volatile oil from various extracts of this plant.

Rai and Thakar (1966) isolated swertiamarin from alcoholic extract of drug treated with ether and ethyl acetate after defating with petroleum ether. They also isolated two homoterpene alkaloids like licoflavina and gentiocremeine and a triterpene sapogenin betulin (m.p. 252-254 °C) from the ether extract, after saponifying it with 5 % sodium hydroxide solution and chromatography of non-saponifiable part over alumina. Various flavonoids like apigenin, genkwanon, iaovitexin, swertinsin, saponarin, glucosyl swertisin and glucosyl isoswertisin were isolated from this plant (Ghoswal et al., 1980; Mabry et al., 1970).

Govindachari et al., (1966) reported that isolated gentianine as an artifact in Enicostemma littorale and suggested that precursor to gentianine- a bitter glycoside swertiamarin or possibly gentiopicroside is present. They also confirmed the presence of swertiamarin by isolating it by chromatography over alumina, and dried residue obtained on evaporation of the methanolic extract of the plant. Six phenolic acids (Daniel and Sabnis 1977), glycoflavones and xanthones are detected in E. littorale (Harborne 1967). Prasad and Bhusan (1954) reported the presence of glycosides and sterols in the petroleum ether extract. Benzene, ether and alcoholic fractions of the drug contain tannins and reducing sugars.
3.4.4 Pharmacological Activity of *E. littorale*

3.4.4.1 Traditional claims

The plant is very bitter in taste, and is used as a stomachic and laxative. It has been employed as a substitute for chirayata (Swertia chirata) and is named as Chota-Chirata. It is also used as an anthelmintic, carminative, and in ‘vata’ diseases in Ayurveda. The whole plant was dried, powdered and given with honey as a blood purifier and in dropsy, rheumatism, abdominal ulcers, hernia, swellings, itchies, filariasis and insect poisoning. The plant has been reported to be used as a type of "Trina Rasa" (Vaidya 1982).

3.4.4.2 Chemotherapeutic activity of *E. littorale*

Steyn (1934) reported *E. littorale* to be safe drug, which gave negative toxicity test in rabbits. It has been reported that the aqueous extracts as well as the dealcoholized extract of the plant are effective against Gram positive and Gram negative organisms. The extracts have been found to be most effective against *S. paratyphi* and fairly effective against following microorganisms in the descending order *E. coli*, *M. pyogenes* var. citreus, *S. typhosa*, *C. diphtheriae*, *M. Pyogenes* var. albus, *M. pyogenes* var. aureus, *S. schottmiilleri* and *B. megatherium*. The active principle responsible for the antibacterial activity was found to be stable against autoclaving (Patel and Trivedi 1957). Ethanol extract of the plant was found to be having antibacterial activity against Micrococcus species and *Shigella dysenteriae* (Natarajan and Prasad 1972). An aqueous extract of *E. littorale* has been reported to possess microfilaricide activity (Kulangara and Subramaniam 1960) and is used for the treatment of filariasis and tapeworm infestation. Decoction of the fresh roots has been used for the treatment of tapeworm infestation in East Africa (Anonymous 1976). Comi (1990) also reported the macrofilaricide activity of the hot water extract of *E. hissopifolium*. 
3.4.4.3 Antipyretic and antimalarial activity

Pills prepared from the aqueous extract along with other ingredients have been known as Mamejjaka Ghanvati and has been recommended for the treatment of different types of fever including Malaria (Ayurvedic Pharmacopoeia, 1966). Dried entire plant of *E. hissopifolium* has been used as a bitter tonic, febrifuge and antimalarial (Natarajan and Prasad 1972). The powdered whole plant of *E. verticillatum* has been used as febrifuge in the doses of 2-3 gm thrice a day with warm water (Anis and Iqbal 1986).

It is reported to be fairly effective against malaria and its administration is not accompanied by any ill effects such as nausea, headache or ringing effects in the ear but it is less effective than quinine and cinchonine alkaloids (Rai 1946). The natives of various areas of northern and center India frequently use the juice of leaves and decoction of the plant in the form of injections to cure malarial and other types of fevers (Dixit and Pandey 1984). Total chloroform soluble alkaloids as their hydrochloride salts showed only a weak antimalarial activity when tested against *Plasmodium berghei* in mice. Its quinine equivalent was in the order of 0.5 when tested by the method of Thurston. Alcoholic extract of the root of *E. hyssopifolium* was screened in vivo and in vitro for antimalarial activity against the NK 65 strain of *Plasmodium berghei*. It was found to possess schizontocidal activity in vivo (58.23 ± 0.41) as well as in vitro (60.13 ± 0.23) at a dose of 1 mg/gk X 4 days and 100 μg/ml respectively. If the plant extracts exhibits only in vitro activity, it may be presumed that the plant material either lacks bioavailability at the site of infection or undergoes biotransformation to yield inactive components. Extract exhibiting exclusively in vivo activity possibly lack direct action on the parasite but is capable of biotransformation to active constituents. But the plant extract of *E. hyssopifolium* exhibiting in vitro and in vivo activities suggests that the active material may possess a direct action against the malarial parasites and the biotransformed products, if any are also active. The LD₅₀ of the plant extract was found to be very high (Misra et al., 1991).
3.4.4.4 Hypotensive action of *E. littorale*

Three alkaloids, isolated from *E. littorale*, were found to possess hypotensive and depressant action on the plain muscles, analgesic action was also observed. The alkaloids were devoid of any anaesthetic action (Sharma and Jain 1960). The alkaloids isolated from *E. littorale* showed marked depression of the heart of the frog and no action on the blood pressure of the dog. Total water-soluble alkaloids did not show any action on the heart of frog and blood pressure of the dog (Natarajan and Prasad 1972).

3.4.4.5 *E. littorale* for the treatment of inflammation and snake-bite

*E. littorale* is found to have anti-inflammatory activity and has been used traditionally for the treatment of inflammation (Sadique et al., 1987; Van 1990). A field survey was carried out among irula tribes and local traditional medical practitioners in Chengalpattu district (Southern India) and it was found that these people crushed the whole plant of *E. littorale* and applied as a paste, locally, for the treatment of snake-bite (Selvanayagam et al., 1995).

3.4.4.6 *E. littorale* for the treatment of leucoderma

A combination of *E. littorale*, *Mussaenda glabrata* (Rubiaceae) and *Psoralea corylifolia* (Papilionaceae) has been used for the treatment of leucoderma by Kani tribes of Trivandrum forests of Kerala, India (John 1984).

3.4.4.7 *E. littorale* for the treatment of veterinary diseases

*E. axillare* has been used as one of the crude drugs for the veterinary practices to cure various important diseases by folklore in Warangal district (Andhra Pradesh) (Reddy et al., 1998).

3.4.4.8 Use of *E. littorale* in the treatment of Diabetes

*E. littorale* is one of the plants that have been mentioned in various ancient Indian literatures to possess antidiabetic activity (Sharma 1991). Tribal inhabitants of North Gujarat are found to be routinely using hot aqueous extract of *E. littorale* for the treatment of diabetes (Shah and Gopal 1985). *E. littorale* is claimed to be antidiabetic when used along with Shilajeet (Trivedi 1969). It has been reported that a 'Phaki'
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(mixture of twelve indigenous plants, of which E. littorale is one of the constituents) showed hypoglycemic activity in diabetic rats. It did not show any hypoglycemic activity in normal rats. Aqueous extract of the 'Phaki' in doses ranging from 50 to 200 mg/kg on I.V. administration in dogs produced a dose dependent reduction in blood sugar (Ainapure et al., 1984). Blood sugar level in anterior pituitary extract induced hyperglycemic rats was found to be decreased when treated with Tribhang Shila, an Ayurvedic formulation, (Zandu Pharmaceuticals Ltd., Bombay), containing E. hyssopifolium as one of the ingredients (Gupta et al., 1962). The antihyperglycemic activity of Tribhang Shila was found comparable to that of tolbutamide (Gupta et al., 1962).

Barot et al., (1975) reported the hypoglycemic effect of fresh juice of the whole plant of E. littorale in diabetic patients. Aqueous extract of E. littorale produced significant reduction in blood sugar in diabetic rabbits. The hypoglycemic effect of E. littorale was suggested to be due to increased peripheral utilization of glucose (Vyas et al., 1979).

Recently the whole plant aqueous extract of E. littorale was reported to decrease the plasma glucose level, glycosylated haemoglobin and glucose-6-phosphatase activity in liver (Vijayvargia et al., 2000).