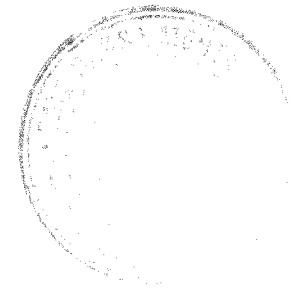


Introduction

Chapter I - Introduction

Ayurveda, the ancient system of medicine of India, depends mainly on plants and their products as drugs. In the ancient texts, the drugs are classified according to their therapeutic action; many of these are considered to be very active, important and are still widely used in practice. The texts of *Ayurveda* contain a full section on *Rasayana*, recommended for enhancement of body's resistance being one of the main therapeutic strategies. In practice, it is believed that various extracts of plant materials release *Rasadhatu* linked to the plasma, and the tissues receive nutrients by selecting required components of this, although ,the exact mechanism still remains elusive. Hence, the quality of *Rasadhatu* is very important, which influences the tissue behavior. The drugs that improve the quality of the *Rasadhatu* and in turn the quality of the entire body are termed as *Rasayana Drugs*[1].

1.1 Quality Control of the Medicinal plants:

Majority of the medicinal and aromatic plants used in the herbal drug industries come from wild collection while the raw materials which are procured from cultivated sources offers consistent quality and lower risk of adulteration. The pharmaceutical industry engaged in manufacturing of herbal products, prefers the standardized extract as active ingredient to be incorporated in formulations. In case the quality of the extracts is not ascertained, the industry has to generate in- house specifications for standardization of such extracts[2].

The term **Standardization** is utmost important for achieving high quality in herbal medicinal products. This expression is generally encompasses to all measures taken during the manufacturing processes, starting from collecting the plant material, associated with quality control in all steps necessary to be taken to ensure homogeneity and efficacy of the product.

Although, plants are used frequently in many traditional systems throughout the globe, their acceptability in modern medicine is remarkably low. The main reason behind this is the lack of standardization parameters[3].

With the exception of pure substances, botanical derivatives obtained from medicinal plants usually contain several classes of compounds endowed with a polyhedral mechanism of action which surprisingly often act synergistically on the same target. The fact makes the process of standardization difficult. The therapeutic potential of a plant can not be assigned to single moiety and presences of various phytoconstituents with different physico-chemical properties provide the matrix a unique status[4].

There are various regulatory requirements proposed by different authorities throughout the globe for standardization and quality control of the herbal products. The process of standardization begins with the identification of the herbal material. The task can be achieved by taxonomic authentification by a professional taxonomist in case of the intact plant material. In case of identification of the plant materials supplied in the dried powder form, various approaches are employed including physical and microscopic evaluation. The Chemo-fingerprinting of the extracts also serve as a tool of confirming both, the identity and purity of any plant material. The presence of different chemical constituents as indicated in the chemo profile provide selection of a few important marker- a chemically defined constituent of a herbal drug which is of interest of control purpose, independent of whether, they have any therapeutic properties or not. Marker may serve as a tool to calculate the quantity of the herbal drugs/ herbal product in the finished product if that marker is determined in the herbal drug or preparation when the starting material is tested. In some guidelines, estimation of the constituents with known therapeutic activity in addition to the chemical markers is also stated. There are certain criterions fixed for selection of the marker component for assessment of the quality of the herbal material/extract like[5],

1. Present in an determinable amount.
2. Specific for the drug, extract or product.
3. Singularly characteristic for a specific ingredient.

4. Analytically accessible.
5. Stable in the particular product.

The methods employed for quantitative estimation of the phytoconstituents are generally based on either of the principle of precipitation, color development or derivatization reactions, sometimes fail to estimate singular component from the complex matrix like extracts unless well associated with separation methods. The methods like High Performance Liquid Chromatography, Gas Chromatography and High Performance Thin Layer Chromatography are few of the examples of such chromatographic methods accepted for routine estimations in the field of Phytochemical analysis due to their efficacy as separation technique.

1.1.1 Development of analytical method for assessment of the quality of herbal raw materials using HPTLC:

The wide varieties of organic substances that are elaborated and accumulated by plants require specific methods for their separation, purification, identification and quantitative determination. One comes across following difficulties while working on plants.

1. The biologically active constituent of the plant is not always clearly defined or generally present as complex mixture of several plant constituents.
2. The active constituent is found as mixture with other ballast compound, generally interferes in determination/estimation.
3. The content of constituents in plants extremely varies hence the proposed methods have be versatile and specific.

The choice of different chromatographic methods can obviously overcome some of above difficulties to achieve the goal.

Although, Gas Liquid Chromatography (GLC) and High Performance Liquid Chromatography (HPLC) are extensively used being sensitive methods for quantitative

determinations of compounds in mixtures, these require tedious pre purifications steps and in most of the cases, lead to loss of constituents of sample also. High Performance TLC, being less accurate, serves as an alternative method because of simple sample preparation methods, even the extract can be employed directly for detection. An important feature of HPTLC is the use of chromatogram as overall fingerprint of crude extract increasing the probability of detecting possible degradation or adulteration of the drug. In addition; many post chromatographic reagents may be exploited to provide compound-specific or class-specific colors or fluorescence in an order to enhance specificity and/or sensitivity.

UV Visible spectrometry, a detection method in HPTLC, can be applied "*In situ*" to check the specificity of the developed mobile phase to resolute the various compounds in the matrix on stationary phase and to determine the optimum wavelength for scanning. The sensitivity of detection although is lower but the capability of method to tackle variable quality of the analyte volume partly overcome this problem too.

Generally, constituent which are present in plant extract at a concentration not lower than 1 % can be quantified without difficulty, when the constituents are fluorescent, these values could be low as 0.05 %. The sample application step is of critical importance for reproducible results. Devices are now available which can automatically apply volumes of 50 nl and more with a precision ofl %. However, in the case of very complex mixtures, this mode of sample application greatly improve the resolution and accuracy of quantification by eliminating the interference of "ballast" constituents or other constituents of interest in addition, a distribution of the compounds within the band is moreover uniform and remains uniform during development.

Retardation factor (R_f) involves the reproducibility of the position of the sample from the lower edge of the plate and the level of the mobile phase in the time. The nature of the solvent used for the sample application, in the best condition, it should not favor the

migration of the constituent around the spot or the band during application and must be at least less polar than mobile phase in order to obtain a pre concentration effect of the constituents in the solid phase and a distribution within this phase which is as small as possible. The problems discussed during the development of analytical method for estimation of the marker component can be eliminated partly by developing the method using HPTLC[6].

Some drugs are described in Ayurvedic system of medicine as *RASAYANAS*, which are basically effective due to their varied type of chemical constituents. These drugs are generally prescribed as restorative, stimulant and as adaptogens to incorporate in many such formulations. Many drugs of this category are yet to be studied for their controversial status and also for the presence of both chemical and therapeutic markers.

1.2 Biological evaluation of Herbal drugs/Extracts:

There is no doubt that most herbs exhibit their effects on a variety of constituents and the idea of synergy within and between them is also gaining acceptance. It is not well-documented in most of the herbal medicines whether they are acting truly in a synergistic way or by additive effects[7]. The factor described generates difficulties in standardization of the herbal drugs. The supportive way to the chemical standardization is to evaluate the plant materials for the claimed therapeutic activity and the results then may be correlated with the set physico chemical parameters. The proposed approach will overcome the inability of physico chemical standardization in explaining the efficacy of the herbal drugs and extracts.

1.2.1 Adaptogenic Potential of the Drugs:

Adaptogens are substances that help organisms to adapt to unfavorable stressful conditions, i.e. physical, biological and mental. They could be of natural origin too. In general, adaptogens work by[8].

1. Supporting the adrenal function, thus counter acting the adverse effects of stress;
2. Enabling the body's cell to have more energy;

3. Helping cells to eliminate toxic by-products of metabolic process;

4. Helping the body to utilize oxygen more efficiently;

5. Enhancing and speeding the proper regulation of biorhythms.

The description of ancient hypothesis of *Rasayana* Therapy and the newly established, currently accepted mechanisms of Adaptogenic drugs are similar and there are many plant drugs indeed which are claimed to be *Rasayana* traditionally and found to be Adaptogenic in pharmacological assessments.

There are so many diseases that are not cured by the new methods of medicine or the medicines available for that diseases are not proper or not effective or having no rationale for prolong use in chronic type of disorders particularly, but it was observed from many experiences that some of the drugs from natural origin are very effective in certain type of disorders

1.2.1.1 Assessment of Adaptogenic Activity of the Herbal Drugs[9]:

Adaptogens are meant to raise the non-specific resistance toward every kind of stress. The basis behind the assessment of adaptogenic activity is

1 Exposure of the animals after pre treatment with the various kinds of the stresses (e.g. physical, chemical, microbial etc.).

2 Testing the Anabolic effectiveness of the drug through hormonal mechanism in various physiological conditions.

3 Testing the changes of brain metabolism after treatment while training the animals for specific tasks.

1.2.1.2 Assessment of Drugs in protecting the animals from chemical induced Stress:

Few of the adaptogenic drugs are hepatoprotective too. They provide the protection to the liver against various types of hepatotoxins[10]. The hepatoprotective potential of the plant extract is assessed by challenging the liver functions using the hepatotoxins like, Carbon

tetrachloride, Thioacetamide, Ethanol, Paracetamol, D-galactosamine etc. The protective/curative effect of the drugs can be assessed by determining few of the vital biochemical parameters, e.g. Serum GPT, Serum GOT, Serum Alkaline Phosphatase, Serum Bilirubin, Serum protein, Cholesterol, Glutathione Reductase, Catalaze and SOD etc. Histological assessment of the liver slides also affords the vital information regarding the potential of the plants/plant extract against such chemically induced stress conditions[11].

1.2.1.3 Investigation of Liver Function[12]:

When the liver is diseased one or more but necessarily all of its functions are impaired. There can be no test for liver functions as whole. The various 'Liver function test (LFTS) are tests of derangements of individual functions of the liver. Since many tests give similar abnormal results in a particular liver disease, it may be possible to extend a conclusion drawn from a single test. The liver biopsy result may not be comparable with LFTS since many functional changes are not mirrored by obvious structural changes in the liver cells.

The routinely performed liver function tests (LFTS) are as follows:

These are based on the following disorders-

A. Abnormalities of bile pigments and bile salts excretion test-.

1. Serum total direct and indirect bilirubin.
2. Urine bile salts, Bile pigments and urobilinogea.

B. Changes in certain enzymes Tests-

1. SGOT (AST)
2. SGPT (AST)
3. Alkaline phosphatase (ALP) and if necessary
4. Gamma – G.T.

C. Changes in plasma proteins Tests-

1. Thymol turbidity test.

2. Determination of total proteins, albumin globulins and A/G ratio.

1.2.1.3.1 Evaluation of Hepatoprotective Activity:

Hepatoprotective activity can be most easily evaluated / screened with the aid of several model systems of liver damage in experimental animals. In all test model systems conditions for liver damage are implemented and an attempt is made to counteract this toxicosis with the substance / preparation under test. The magnitude of the protective effect can be measured by estimating the enzyme activities and the rate of survival and can be verified histologically. The available methods are *in vivo* and *in vitro* methods

1.2.1.3.2 Experimental models for Hepatoprotective screening:

Several chemical reagents and drugs which induce liposis, necrosis, cirrhosis, carcinogenesis and hepatobiliary dysfunctions in experimental animals are classified as hepatotoxins. The most important ones used are Carbon tetrachloride (CCl_4)[13], Thioacetamide (TAA) [14], D-galactosamine[15], Paracetamol[16], Chloroform[17] and Ethyl alcohol[18].

Aldehydes are generated as a result of lipid hydro peroxidation. It has been established to be causally involved in such tissue injuries generally hepatotoxicity due to different type of chemicals. The hepatotoxicity is well correlated with the depleted level of antioxidant in living system.

1.2.1.3.3 Hepatoprotective medicaments[19]:

Hepatoprotective medicaments are generally classified into 3 categories without any strict delineation among them

1. Anti hepatotoxic agents:

These generally antagonise the effects of any hepatotoxin causing hepatitis or any liver disorder or disease.

2. Hepatotropic agents:

These generally support or promote the healing process of the liver. In practice these two activities can not be easily distinguished from each other.

3. Hepatoprotective agents:

These generally prevent various types of liver affections prophilactically.

In general any hepatoprotective agent can act as an antihepatotoxic or hepatotropic agent but the vice versa is always not true.

1.2.1.3.4 Plant drugs used in liver disorders [10]:

There are number of phytoconstituents from plants which have exhibited antihepatotoxic activity. Some of the crude drugs with activity against liver diseases are:

Eclipta alba (Asteraceae),

- Glycyrrhiza glabra (Leguminosae),
- Boerhaavia diffusa (Nyctaginaceae),
- Phyllanthus amarus (Euphorbiaceae),
- Silybum marianum (Compositeae),
- Uncaria gamber (Rubiaceae),
- Andrograhis paniculata (Acanthaceae).

Some of the reported constituents with pharmacologically / therapeutically proved claims may be enlisted as'

- Silymarin, Glycyrrhizin,
- (+) –Catechin,
- Saikosaponins,
- Picrolive etc.

1.2.2 Lactation and Herbal Drugs:

There are so many plants traditionally recommended in human to induce the lactation after child birth. *Asepragaus racemosus, Caraca Papaya, Leptadenia reticulata, Foeniculum vulgare, Trigonella spp.* are few of the herbs which are used to enhance the lactation. These plants are incorporated in herbal lactogenic formulations too.

1.2.2.1 Anatomy and Physiology of mammary glands[20]:

All the species studied, the morphology of the mammary gland is both simple and relatively homogeneous. Glandular tissues are classified in two broad classes as parenchyma and stroma. In the developed gland, the milk is synthesized in secretory (epithelial) cells, which are arranged in single layers lining the central cavities of spherical structures. Milk secreted into the alveolar lumina is drained via a system of arborizing ducts towards the body surface. The secretory elements of parenchyma are referred to as the lobulo-alveolar system in contradistinction to the duct system. Surrounding each alveolus, adjacent to the base of the secretory cells, are numerous branching, spindle shaped, myoepithelial cells. These cells contract in response to increased blood concentrations of oxytocin and forcing milk from the alveolar lumina to the ductal system.

1.2.2.2 Endocrinology of Lactation:[21]

The anterior pituitary contains specific hormones responsible for the initiation of lactation, of which Prolactin appears to be the important hormone. Prolactin causes a localized initiation of milk secretion when injected into the rabbit mammary gland. In addition to prolactin, adrenal corticoid hormones are required for the initiation of lactation in most animals. In one strain of mouse, somatotrophin (STH) can replace prolactin in the initiation process. In the rabbit, prolactin alone can initiate lactation in the hypophysectomized animals.

The prolactin secretion and/or release by the pituitary gland is controlled by the hypothalamus and by a direct action of compounds on the pituitary gland. The hypothalamus

produces a chemical compound, prolactin inhibiting factor (PIF), which inhibits the prolactin production and/or release from the pituitary. Pituitaries transplanted to other parts of the body or grown in organ culture without the hypothalamic extract increase the release of prolactin. Compounds, such as reserpine, epinephrine, acetylcholine and the suckling stimulus exert their effect by decreasing the hypothalamic content of PIF. Estradiol appears to work in the same way, as well as having a direct stimulatory effect on the pituitary gland.

The maintenance of milk secretion is dependent upon the removal of the milk and a suckling or milking stimulus in most animals. The suckling stimulus is involved in the release of prolactin, ACTH, and oxytocin from the pituitary gland.

Dopamine, acting centrally, binds with dopaminergic receptors in anterior pituitary and stimulates the secretion of PIF thus suppresses the established lactation by lowering the Prolactin release. Antidopaminergic drugs like, Domperidone and Metaclopramide are used clinically to induce the lactation in post pregnant patients.

1.2.2.3 Biochemistry of Lactation: [21]

During the phase of the lactation the mammary glands are highly active biochemically. The mass of mammary glands increases as a storage of protein, while the fat content of the mammary glands is decreased. The epithelial cells under the stimulus of the Prolactin secrete the milk in alveolar spaces thus the alveolar spaces are filled with the milk. DNA and RNA content of the mammary gland are also increased during the lactation phase as compares to the resting mammary glands. The glycogen content is also increased significantly as the reserve of the energy necessary for the metabolic processes.

1.2.2.4 Assessment of Lactogenic Potential:

In vivo assessment of the lactogenic potential of the drugs may be performed as per the approaches described below: [21]

1. Assessment of Biochemical Parameters which are altered during the lactation, e.g. estimation of total protein and glycogen content of the mammary glands tissue, estimation of mRNA content of the mammary glands which are involved in synthesis of particular components of the milk.
2. Quantitative histological assessment of the mammary glands tissues
3. Hormonal assessment, e.g. estimation of serum Prolactin.

1.3 Controversial Drugs in *Ayurveda* system of Medicine:

Ayurveda utilizes mainly plants as drugs, although, many of these plants still lack standards for identification of correct sources thus generate controversy. Often, the plant material provided of a particular botanical source does not possess claimed therapeutic activity or sometimes plant supplied has no resemblance whatsoever with the actual one. This type of controversy generally is encountered in case of extensively used plants.

The original description of plant materials is available in the texts written in *Sanskrit* having many synonyms for one plant drug. On latter dates *Nighantus,* appeared being simplified versions containing monographs with therapeutic and morphological descriptions as explanatory volumes [22]. Although, several such volumes are available practically dating of every era of history, still in present days there are drugs considered as controversial drugs. In simple terms, these drugs have more than one botanical source and it is rather difficult to choose the correct one even from the available description. Thus a situation arises to place such drugs having controversial source as far as identification is concerned[23].There are various reasons responsible for development of identification controversy in traditional drugs[24,25].

1. In *Vedas* and in *Samhitas,* detailed information on selected plants is written in complicated manner generally too difficult to understand by common users. At this point of time *Nighantus* came into the picture. These are compilations prepared by

local practioners of the region, based on the information from *Veda*, inscribing synonyms, morphological descriptions, vernacular names and pharmacological actions. *Nighantus* could be considered as dictionaries of ayurvedic drugs with local impacts on description, at times, complicating the identification process. Ironically, the description in *Nighantus* also became one of the causes of generating controversy due to difficulty in distinguishing original plant as the descriptions are similar to many other plants. Moreover, *Nighantus*, are fully influenced by local impact of different geographical region.

2. Most of the ayurvedic texts are written in *Sanskrit*, which generally is not a spoken language of common man. One word is usually used for different articles to name them, like word *Arka* means Sun, a Mantra (Hymns), a food article, name of a plant too and under this any of these items are described. This practice makes identification a very difficult task. In *Nighantus*, also similar practice is followed.
3. *Niruktas*, like *Nighantus*, are available compilations, which provide meanings of such words and generally referred also. There are as many minimum of 18 N*iruktakars* (authors) of Niruktas, mentioned in history of Sanskrit literature and they described various terms in different manner in their own way, this has again formed as one of the reasons for development of controversy.
4. The original descriptions are available on *Bhoj-parta* or *Tal-patra*, and different men were entrusted to copy those writings. The mistakes they have committed in doing so have been carried forward through generations thus led to controversy of today.
5. With passage of time Sanskrit was replaced by local languages and assigning name to various plants posed a great problem because a particular plant had different names in different language of different area.

6. In *Ayurveda* texts the morphological descriptions of plants are not available in scientifically accepted manner and plants are misidentified due to similarity in appearance.
7. Physicians of Vedic era came in contact with people of other civilizations thus some new words in Indian literature were introduced in different part of the country, which later on caused controversy in correct identification, for instance, *Kshauma* is the name of material used as bandage; the original word *Kshau- ma*, is of Chinese origin meaning fiber from *Kshau* tree with passage of time, *Atasi* was started to be used as a source of material for bandage and became synonym of *Kshauma* in some *Ayurveda* books, although both are different plants.
8. Another reason for development of such controversy is incorrect interpretation of information of authentic texts, where each word has a typical meaning.

Information related to, chemical as well as clinical uses of medicinal plants in traditional and folklore systems of medicine provide scope of further investigations. The following plants were selected on basis of their extensive use in the traditional systems of medicine as *RASAYANAS*[26], generally used as adaptogenic, remedy for disorders of reproductive system etc[27].These plants are described under the category of controversial plants being commonly sold in the market in the singular name of *JIVANTI* although belongs to three different genera.[28, 29].These plants are extensively used in dairy cattle feed to increase the production of milk, so much so that one of these have reached to the level of extinction.

1. Whole plant of ***Leptadenia reticulata***
2. Whole plant of ***Dregea volubilis***
3. Whole plant of ***Pentatropis microphylla***

All belonging to family *Asclepiadaceae,* were selected for present studies.

1.4 OBJECTIVES SET FOR THE STUDIES:

In order to address issues arising out of the controversy in sources for extensively exploited plant ***Leptadenia reticulata*** as lactogenic in dairy cattle feed, so much so that it reached to the level of extinction, the following objectives for the present studies were set.

1. To identify and develop standards for ***Leptadenia reticulata,*** mentioned and also its substitutes[30] ***Dregea volubilis*** and ***Pentatropis microphylla*** all sold as ***'Jivanti'*** as ***Rasayana*** drug of Ayurveda.
2. To detect the compounds from the different extracts as markers.
3. To screen some of the extracts from these plants for their endowed biological activity.
4. Development of analytical methods for assessment, and standardization of extracts /formulations of the selected plants using available markers.

1.6 Review of Literature:

Jivanti, as per Ayurveda is described as one of the *Rasayana* and Rejuvenating drugs, considered as *sheet (cool), snigdha* and *laghu*, having *sheet virya* and *madhur vipaka* [29]. The plant is used to treat leprosy, tuberculosis and very useful in maintaining and curing diseases related to vision. It is considered as an important remedy in treatment of Asthma, as purgative and is used to treat mercury poisoning [26]. Due to variety of uses, *Jivanti* forms an important drug in Ayurvedic system of medicine described in *samhitas* and in almost in all the *Nighantus*. The plant is known for beneficial effects on development and maintenance of reproductive system in male and female and in treatment of habitual abortion traditionally [31]. Different plants in different region of India are used with more or less similar description on *Jivanti*. Obviously physicians of a specific region followed for identification books written by the scholars of same region and thus such an important drug became an item of identification controversy, as it has got more than one botanical source.

In central and western part of India *Leptadenia reticulata* (*Asclepiadaceae*) is used as *Jivanti,* while in North India and in Eastern India, *Cimicifuga foetida* (*Ranunculaceae)* and *Desmotrichum fimbriatum* (*Orchidaceae*) are used as *Jivanti*[29].

In South India, a morphologically similar plant to *Leptadenia reticulata,* named as *Holostemma annulare (Asclepiadaceae)* is used as *Jivanti*[29]. Some scholars also consider *Marsdenia volubilis (*syn. *Dregea volubilis*) as a type of *Jivanti*[28].The reports suggest that at one point of time plant of *Sarcolemma brevistegma* (*Asclepiadaceae*) was also considered as *Jivanti*[32].At present in Gujarat, retail suppliers provide stems of *Tylophora fasciculata* as bitter *Jivanti*, almost all different therapeutic properties than *Jivanti*[30]. Due to lack of information on identification and also local impacts it has become very difficult to select right plant as *Jivanti.* Some suppliers provide different plants under the name of *Jivanti* irrespective of therapeutic actions and toxicological effects in therapeutics.

Nevertheless, morphological descriptions reveal that if *Jivanti* of Ayurveda belongs to family *Asclpeadaceae,* then *C. foetida* of *Ranunculaceae* can not be considered as *Jivanti.* Another plant *S. brevistigma* (*Asclepiadaceae)* known as *Jivanti*, is now identified as *Somvati Lata* of *Aryas* [29].

Ayurvedic Formulary of India suggests *Leptadenia reticulata* as *Jivanti*[33]. The available information regarding phytochemical and pharmacological status of the plants selected is mentioned here.

1.6.1 Whole plant of *Leptadenia reticulata* (*Asclepiadaceae*):

The drug is known with different names in different parts of India as;

Hindi	*Dori*
Gujarati	*Dori*
Marathi	*Khirkhodi*
Sanskrit	*Arkapushpi, Jivanti*
Tamil	*Palaikodi*
Telgu	*Kalasa*

Fig: 1.1 Aerial Parts of *L.reticulata* (Family: *Asclepeadaceae*)

L.reticulata is a twinning shrub with corky deeply cracked bark and numerous branches. The younger branches are terete, glabrous or hoary-puberulous. Leaves are thinly coriaceous, ovate, and acute which are glabrous above, more or less finely pubescent (especially on the nerves). Base of the leaves is rounded or subcordate (rarely subacute), petioles are 6-20 mm long and puberulous. Flowers are greenish-yellow and in lateral or subaxillary, many – flowered hoary-puberulous globose cymes are present. peduncules are arised from between the leaves or subaxillary, sometimes in pairs and are pubrulours with 3-4 mm long , pubreulous pedicels. Calyx is pubescent outside and divided to about the middle with 1.25 mm long segments, ovate oblong subacute in shape. Corolla is 5 mm long (about 8 mm across when expanded), tube is very short and glabrous; lobes of the limb are thick, 2.5 mm long, ovate-oblong, sub obtuse, with revolute markings, pubescent on both surfaces. Coralline corona of 5 quadrate truncate fleshy lobes is present at the sinuses. Staminal corona is minute, annular, close to the staminal columns. Anthers are without membranous appendages and incumbent on the style-apex, pollen masses are ovoid, large, waxy and pellucid at the apex. The pollen masses are attached to the minute pollen-carriers by moderately long caudicles; follicles are subbing woody, 6.3-9.0 cm long, turgid and tapering into an obtuse shortly curved beak. Seeds are 6 mm long, narrowly ovate-oblong, acute and com is 3.2-3.8 cm long. Tribes utilize this plant as stimulant and tonic[34].

Microscopic studies of the roots of *L.reticulata* revealed the presence of thin walled and cuboidal epidermal cells followed by cortex. Cortex is made up of parenchymatous cells along with stone cells. Endodermis is distinguished clearly by the presence of capsparian dots. Interxylary phloem is embedded in wood portion in form of islets. The sclerids are of oval shaped.

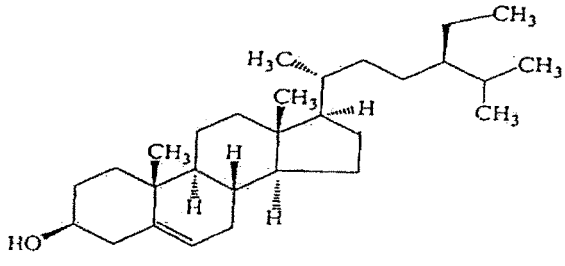
The epidermal cells of the stem are elongated and converted in the multicellular trichomes. Parenchymatous cells and pericyclic fibres are present in cortex region. The phloem is made up of 2-3 layers of thin cells. Intraxylary phloem is present in the periphery of the pith. The pith consists of thin walled isodiametric parenchymatous cells.

The transverse section of leaf showed isobilateral arrangement of spongy parenchyma and palisade cells in laminal portion. The midrib portion consisted of the layers of collenchymatous cells after the epidermal cells. Three bicolateral vascular bundles are observed. The central one is larger and crescent in shape while other two are smaller and circular. The anomocytic stomatas are seen. The covering trichomes are uniceriate, multicelluar and collapsed cell type[34].

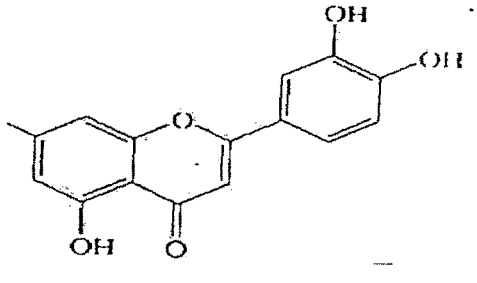
Whole plant of *L.reticulata* is found to contain considerable amount of sterols, of which γ-sitosterol could be identified[35]. Aerial parts of *L.reticulata* were found to be devoid of alkaloids, flavonoids and saponins[36].

Pet ether extract of leaves and twigs of *L.reticulata*, yielded hetriacontane, α-amyrin, β-amyrin, stigmasterol and sitosterol[37],where as acetone and methanol extract gave flavanoids-diosmentin, and luteolin[38].Three flavanoid glycosides viz. isoquercitrin, rutin, hyperoside and quercetin were isolated from alcoholic extract of pericarp of follicles[39] . Tocopherols were also found to be present in stems of *L.reticulata* [40]. One novel type of sugar was also isolated from stems of *L.reticulata* [41].

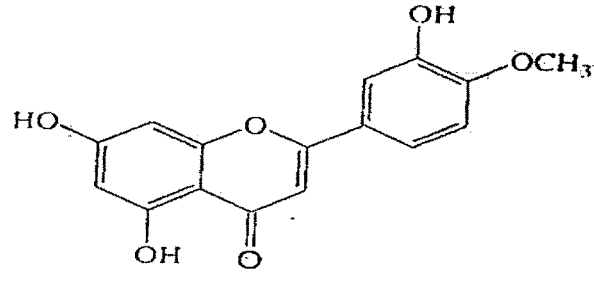
Analytical methods are reported to estimate Rutin, Tocopherol acetate and Stigmasterol from the plant extracts[42, 43].



β-Sitosterol



Luteolin



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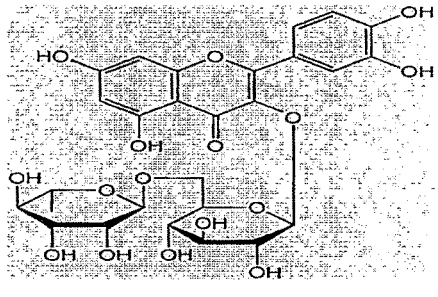
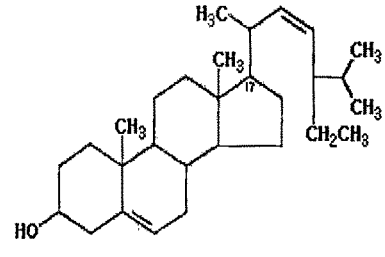


Rutin



Stigamsterol

Dried plant powder and different extracts of the plant were studied thoroughly to evaluate actions on physiological functions *in vivo* and *in vitro*. Clinical studies were also performed to access the effects of herbal remedies containing *L.reticulata* as one of the ingredients. Stigmasterol and Ether fraction of *L.reticulata* were found to possess lactogenic activity as assessed by selecting the parameters like pup weight, height of the mother rats, secretary rating, parenchyma percentage, estimation of glycogen content of abdominal mammary gland and the protein contents of the pectoral mammary glands. Isolated stigmasterol content was

found to be more active as compare to the whole extract[44]. Stigmasterol and non saponifiable fraction from *L.reticulata* showed estrogeniomimetic activity in cycling female rats[45]. The aqueous extract of stem of *L.reticulata* showed highly prolonged hypotensive action in anaesthetized dog, the initial hypotension being followed by a complete recovery and secondary progressive hypotension. It was concluded that extract has no parasympathomimetic or adrenolytic actions but it has capacity to block the pressure response to nicotine. The extract showed transient negative inotropic and chronotropic actions on dog's heart. Extract was also found to possess slight respiratory depressant and bronchoconstrictor effect. Pet ether and chloroform extract possess no action on heart but alcoholic extract produced hypotensive action[46].

Aqueous and ethanolic extract of *L.reticulata* roots and of aerial parts showed antibacterial activity against *Mycobacterium pyogenes* var. aureus, *Mycobacterium pyogenes* var. albus, *Mycobacterium* pyogenes var. citreus, *Mycobacterium* pyogenes var. β-mefgatherium, *Streptococcus pyogenes* var. α-haemolyticus, *Streptococcus pyogenes* var. β- haemolyticus, *Salmonella typhi, Salmonella paratyphi, Salmonella schottmulleri, Escherichia coli, Pseudomonas vulgaris* and *Pseudomonas pyocyanea.* The aqueous and 50% alcoholic extract of both leaves and roots of *L.reticulata* showed antifungal activity against *Trichophyton rubrum*[47].

The claims regarding spermatogenesis activity of the herbal formulation was assessed in mice and the spermatogenetic response was observed. The indigenous drug SPEMAN containing *Leptadenia reticulata* as one of the ingredient showed and anabolic-cum-androgen like activity on accessory reproductive functions of mice[48].

Leptadenia reticulata is used as one of the ingredients in many *Ayurvedic* formulations including *Chyavanprash, Manasamitra Vatakaaaa, Anu Taila, Sasdabindu Taila , Ashoka Ghrita, Jivantayadi Ghrita, Amrta prasa Kvatha* etc[49]

1.6.2 Whole plant *of Dregea volubilis* (*Asclepiadaceae*):

The drug is known in vernacular languages in India as;

Hindi	*Dori*
Gujarati	*Moti Dori*
Marathi	*Khirkhodi*
Sanskrit	*Arkapushpi, Jivanti, Madhumalti*

Fig: 1.2 Aerial Parts of *D.volubilis*(family: *Ascepeadaceae*)

Morphological description:

A twining ash-colored shrub with very long, glabrous older branches often having lenticels, small black dots, while young branches are green, slender and smooth. Leaves are 6.3 – 15.00 × 4.5- 11.5 cm and broadly ovate or sub orbicular, acuminate, glabrous or more or less softly pubescent, reticulate veinsand with a few small glands just above the petiole. Base is rounded or cordate; petiole is 1.3-3.2 cm long.

Flowers are numerous, green or yellowish green, in lateral droopling. They are umbellate cymes, peduncles arise form between the petioles. Calyx is divided nearly to the base, segments are about 2.5 mm long, ovate-oblong, obtuse or sub acute, ciliolate. Corolla is 6 mm long, deeply divided, glabrous outside, lobes are 5 mm long, broadly ovate, obtuse, veined, overlapping to the right, corona lobes are large, fleshy, the upper free portion is rounded on the outer edge, obliquely truncate at top and with a small appiculation is present on the inner edge, which lies against the top of the anther. The staminal column arises from

the base of the corolla, and anther tips are membranous, broadly ovate-oblong, obtuse. Follicles are 7.5 – 10 cm long. Seed are broadly ovate, 13×8 mm, flattened, strong margined, pale yellowish brown and coma is 4.5 cm long and copious. There are two varieties available one is termed is sweet variety while another is termed as bitter variety. The sweet variety is used as tonic, coolent and aphrodiasiac, cures vata and burning sensation and it is used in treatment of the diseases of eyes by tribes. These are some of the indications for which *Jivanti* is prescribed in *Ayurveda*[50].

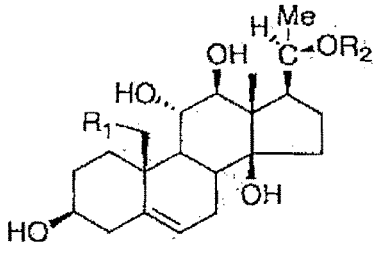
Microscopic studies of the roots of *D.volubilis* revealed the presence of ovular celled epidermal layer followed by cortex consists of stone cells, arranged in groups. The endodermis is not easy to distinguish. Interxylary phloem is absent.

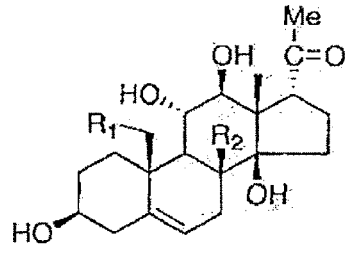
The TS of stem consisted of single layered epidermis along with multicellular trichomes. Collenchymatous cells are present in cortex region followed by 1-2 layers of pericyclic fibres. The phloems are radially elongated. Interxyllary pholems are collapsed with pith cells and are usually not observed.

Blunt, uniceriate, multicellular covering trichomes are seen in transverse section of leaf. The laminal portion looks dorsiventral due to presence of only one layer of palisade cells at ventral side. There are 3 vascular traces are present. The central one is larger while other two are smaller and circular in shape. The stomatas present are of anomocytic type[28].

The seeds of *D.volubilis* possess ester glycosides with sterol genins while stems and leaves showed presence of taraxerol and taraxerol benzoate. The presence of two steroidal compounds, kaempferol along with other unknown phytoconstituents is also reported. Dregiogenin A, d-cymanose, l-oleandrose and draveogenin have also been isolated [51, 52]. The bark of *D.volubilis* revealed presence of kaempferol and kaempferol 3-galactoside and some unidentified glycosides with pregnane aglycones[53]. Different extracts of flowers of *D.volubilis* showed presence of volubiloside A, B, C and new polyhydroxy pregnane

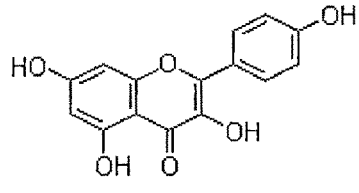
glycosides dregelol, volubilogenone and volubilol along with pregnane derivatives-drevogenin, isodrevogenin and 17- alpha-marsdenin[54].





1. Dregealol, R_1 = H; R_2 = tigloyl
3. Volubilol, R_1 = OH; R_2 = H
6. Drevogenin D, R_1 = R_2 = H

2. Volubilogenone, R_1 = OH; R_2 = H
4. Iso-drevogenin P, R_1 = R_2 = H
5. 17α-Marsdenin, R_1 = H; R_2 = OH



kaempferol

The glucosidic fraction isolated from the aerial parts of *D.volubilis* was found to exert stimulant action on all the organs of body having cholinergic nerve supply and causes prolong fall in blood pressure of anesthetized dog[55].

The fresh leaf juice of *D. volubilis* is believed in folklore as effective in controlling diabetes mellitus. The scientific study was carried out to evaluate alcoholic extract of the leaves for antidiabetic effects which however was not found to be effective in decreasing the blood sugar level while juice of leaves was found to be effective [56]. The plant contains number of pregnane and steroidal derivatives, known for their anti fibritic activity, and the plant is utilized by tribes of central and eastern Asia as a remedy against fibritic disorders[57].

Hemjivanti taila is one of the formulations which are used to treat contusions, fresh wounds and fractures. Another one is *Murivenna taila* also used for the same purposes and when both *tailas* were evaluated for presence of phytoconstituents it was found that they contained three

similar phytoconstituents, one of them was isolated and it was found to be Teraxerol. It was concluded from these experiments that those three phytoconstituents may have major role in therapeutic activity of selected formulations [58].

Aqueous extracts of mature leaves of *D. volubilis* were administered orally under light ether anesthesia to male Sprague-Dawley rats for 14 days. Key hepatic enzyme concentrations and histopathological changes in the liver in treatment groups at the end of 14 days suggested that selected plant extract exerted no hepatoprotective activity. One of the parameters that can be used to evaluate the adaptogenic activity is protection provided by the drug to hepatic functions in presence of shock conditions applied to animal. Thus results obtained are considered as one of the negative reports as far as the adaptogenic activities are concerned [59].

1.6.3 Whole plant of *Pentatropis microphylla* (*Asclepiadaceae*):

The plant is known different vernacular names in the different region of contry.

Gujarati: - Shiagroti, singroti, Surjavel

Hindi: - Ambarvel

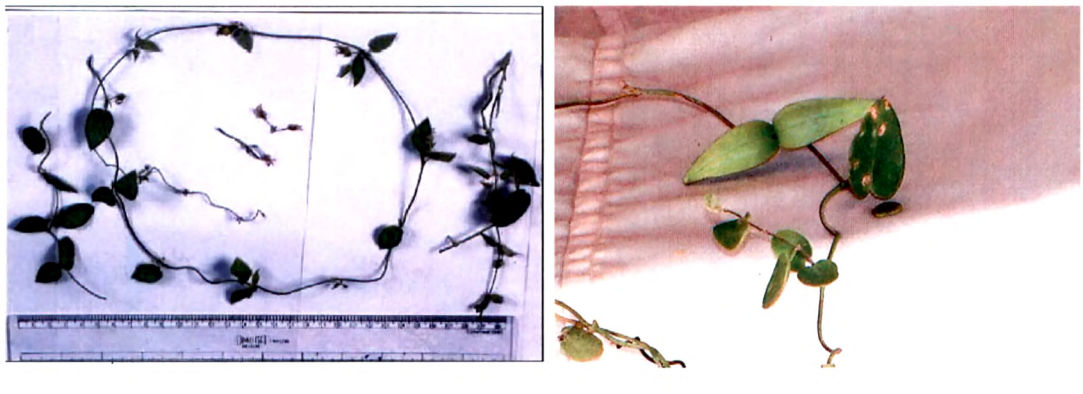
Malayalam:- Parpparam

Marathi: - Shingrota

Sanskrit: - Shringariti, Suryavalli, Kakanasa

Tamil: - Pulapala

Fig; 1.3 Aerial parts of *P.microphylla* (Family: *Asclepeadaceae*)



In Gujarat plants of *Pentatropis spiralis* or *P.microphylla* (Asclepiadaceae) are available under tribal name as *Kauaroti* or *Dodi*[60] .These bear fruits which resemble in shape with the beak of a crow, and hence termed as *Kaknasa* of Ayurveda. This plant is also used as pot herb like that of *Jivanti*. Ayurveda mentions *Pentatropis spiralis* (*Kaknasa)* to be useful in treatment of diseases of reproductive system. An authoritative book on Ayurveda in regional language mentions the plant known as *Kaknasa* is another type of *Dodi* and even many people consider this plant is as *Jivanti*[32]. Aqueous extract of roots is used in treatment of Gonorrhea[61].

P.microphylla is a twining glabrous perennial herb, often slightly woody at the base. The stems are very slender. Leaves are 1.3 to 2.5 cm long broadly oblong or ovate-elliptic, obtuse, mucronate. The base of the leaf is rounded or cordate. The petioles are 4-6 mm. long. Flowers are arranged in lateral few-flowered cyme; peduncles is 3-2.5 mm. long or less and pedicels are filiform, 13 mm. long. The buds are sub-globose; calyx is deeply divided and lanceolate in shape. Corolla is divided almost to the base and lobes are 5 mm. long, lanceolate in shape.[60]

Microscopic studies of the transverse secions of the root revealed presence of thickend, elongated and barrel shaped epidermal cells. The cork cells are rectangular in shape. Cortex is made up of parenchymatous cells. Xylem cylinder is intruded by three or four wedge shaped patches of parenchymatous cells resulting in stellate shaped xylem cylinder. Phloem is present in the form of a ring interrupted by the introduction of parenchymatous cells.

Stem consisted of thick walled and compactly arranged 2-3 layers of the epidermal cells. The cork cells are radially elongated and thin walled. Cortex is homogenous and made up of rounded parenchymatous cells arranged loosely with small intracellular spaces. Xylem vessels are arranged in cylindrical shape. Primary xylem vessels are arranged in form of a vertical raw. Phloems are bicolateral and intraxyllary phloem patches are seen towards inside

the pith portion. The leaves are bicolateral and trichomes are absent. Stomatas are paracytic and anomocytic[62].

Different extracts of the aerial parts of the plant showed presence of octacosenol, alpha-amyrin, friedelin, beta- sitosterol, salicylic acid and some unidentified triterpenoids. [63]

1.7 Research Envisaged:

The review of literature revealed that a number of constituents are reported from different parts of the plants and the plant extracts are evaluated for different physiological actions. These plants with such controversial status have never been subjected to the comparative screening either chemically or biologically. The scientific data for their identification are yet to be generated and their utility as lactogen and adaptogen is yet to be established. Present studies were, therefore, planned to achieve the said objectives on the following lines:

1. Pharmacognostic studies
    - Morphological studies
    - Microscopic studies of powdered drug
2. Comparative Phytochemical studies of the plant extracts
3. Development of Parameters for standardization
    - Development of WHO standards
    - Quantitative Determination of various Inorganic ions in plant materials
    - HPTLC finger printing profile of Methanolic extracts
    - Detection of different marker compounds from plant extracts
    - Development of methods for detection and estimation of some markers in selected plant materials
    - Development of stability profile of extracts
    - Evaluation of different marketed samples of *L.reticulata.*
4. Evaluation of Ayurvedic formulation –*Jivantyadi Ghrit*

5. Biological assessment of different extracts of the plants selected

    1. Assessment of Adaptogenic potential of extract of the selected plants

        - Assessment of *in vitro* antioxidant potential of the plant extract.
        - Evaluation of Hepatoprotective potential of plant extract *in vivo* and *in vitro*
        - Assessment of comparative adaptogenic potential shown by methanolic extracts in swim endurance test.

    2. Assessment of lactogenic potential

        - Assessment of lactogenic potential of extracts of *D.volubilis* and *P.microphylla* in lactating rats and comparison with extracts of *L.reticulata.*
        - Assessment of Lactogenic potential of the formulations prepared from Pet.ether extract of *L.reticulata*

6. Development of software to arrange and retrieve the generated data pertaining to medicinal plants.