1.1 SPLENOMEGALY

The spleen is the largest lymphoid organ in the body. One of its primary functions is to filter defective and/or foreign cells. It is a reserve for blood storage. It is also able to form red cells and other components of the blood when the bone marrow is atrophied, as in extramedullary erythropoiesis. More important, it is involved in the destruction of old or damaged red cells and platelets. Finally, it hypertrophies to fight infection just like the lymph glands\(^1\).

The spleen is located in the upper left corner of the abdomen. It is bordered by the body organs that are closest, which are the stomach, left kidney and the top of the colon. It is normally protected by the rib cage. However, in cases of severe trauma to the chest or abdomen, the spleen can be injured by the overlying rib cage. In some infections (mononucleosis) or in some disease states (blood diseases like hereditary spherocytosis or cancers like leukemia and lymphoma) the spleen can become enlarged. Then it may come down into the abdomen away from the protective ribs and may be vulnerable to injury.\(^2\)

![Figure 1.1: Position of spleen and ribs in abdomen](image)

The body of the spleen appears red and pulpy, surrounded by a tough capsule. The red pulp consists of blood vessels (splenic sinusoids) interwoven with connective tissue (splenic cords). The red pulp filters the blood and removes old and defective blood cells. The white pulp is inside the red pulp, and consists of little lumps of lymphoid tissue. Antibodies are made inside the white pulp. Similar to other organs of the lymphatic system, particular immune cells (B-lymphocytes and T-lymphocytes) and blood cells are
either made or matured inside the spleen. Blood enters the spleen via the splenic artery, which subdivides into many tiny branches. Each branch is encased in a clump of lymphocytes, which means every drop of blood is filtered for foreign particles as it enters the spleen.³

![Diagram of spleen sections]

**Figure 1.2: Transfer section of spleen showing internal tissues of spleen**

Various functions of the spleen are:

- Helps in immunity (protection against infection)
- Stores blood for the body and releases it when needed
- Destroys bacteria
- Destroys worn out and damaged platelets
- Destroys worn out and damaged red blood cells

Splenomegaly is an enlargement of the spleen beyond its normal size. The spleen is located on the left side of the abdomen and weighs around 200g (8 oz) in the average healthy adult. The spleen can be considered a dual-purpose organ: it filters the blood and removes abnormal cells (such as old and defective red blood cells), and it makes disease-fighting components of the immune system (including antibodies and lymphocytes). Since the spleen is involved in so many bodily functions, it is vulnerable to a wide range of disorders involving the blood or lymph system, and by infection, malignancies, liver disease, or parasites.⁴
Splenomegaly is usually caused by systemic disease and not by primary splenic disease. It is usually caused by infection (excessive antigen stimulation), autoimmune disorders (disordered immunoregulation), or hemolysis (excessive destruction of abnormal blood components). Because of exposure below the protective rib cage, splenomegaly results in increased risk of splenic injury or rupture.

1.1.1 Various causes of splenomegaly

(1) Infection/inflammation
- Acute hepatitis (B or C)
- Viral (EBV, CMV, HIV)
- Bacterial (SBE, cat-scratch disease, TB, histoplasmosis, toxoplasmosis, *Salmonella*)
- Systemic lupus erythematosus (SLE)
- Rheumatoid arthritis
- Inflammatory bowel disease
- Celiac disease
- Acidosis
- Chronic granulomatous disease
- Serum sickness
- Protozoal infection (malaria and schistosomiasis are rare in the U.S.)

(2) Hemolytic anemias
- Hereditary spherocytosis
- Hemoglobinopathies
- Thalassemia major
- Nonspherocytic hemolytic anemias (pyruvate kinase deficiency)

(3) Malignancy
- Leukemia, 50% of children with ALL
- Hodgkin disease, non-Hodgkin lymphoma
- Metastatic disease
(4) Extramedullary hematopoiesis
- Thalassemia major
- Osteopetrosis (rare)
- Myelofibrosis

(5) Storage/infiltrative disorders
- Histiocytosis
- Lipidoses (e.g., Niemann-Pick, Gaucher)
- Mucopolysaccharidoses (e.g., Hurler, Hunter)

(6) Congestive
- Chronic congestive heart failure
- Portal hypertension
- Portal or splenic venous thrombosis
- Hepatic fibrosis
- Cirrhosis

(7) Structural
- Hematoma (trauma)
- Cysts or pseudocysts

(8) Wandering spleen

1.1.2 Approach to the Diagnosis
There are several clinical clues. One looks in the physical examination for jaundice, lymphadenopathy, a rash, sore throat, hepatomegaly, and a positive Rumpel–Leede test. The combination of symptoms and signs will eliminate certain causes and make others more plausible. For example, splenomegaly with jaundice but no hepatomegaly suggests hemolytic anemia. The size of the spleen is also an important differential feature. If the spleen is very large, it should suggest myeloid metaplasia, chronic myelogenous leukemia, Gaucher disease, and kala-azar.

1.1.3 Laboratory Tests for splenomegaly

1. CBC and differential (anemia, leukemia)
2. Blood smear for morphology (anemia)
3. Reticulocyte count (hemolytic anemia)
4. Platelet count and clot retraction (thrombocytopenia)
5. Radioactive chromium–tagged red cell (hemolytic anemia)
6. Serum haptoglobins (hemolytic anemia)
7. Bone marrow examination (aplastic anemia)
8. Blood cultures (SBE)
9. Febrile agglutinins (infectious disease)
10. Heterophil antibody titer (infectious mononucleosis)
11. Brucellin agglutinins (brucellosis)
12. Blood smear for parasites (malaria, trypanosomiasis)
13. Liver function studies (cirrhosis, Banti syndrome)
14. RA test (Felty syndrome)
15. ANA test (collagen disease)
16. Serum protein electrophoresis (lymphoma, collagen disease)
17. Hemoglobin electrophoresis (hemolytic anemia)
18. Esophagram (esophageal varices) (portal cirrhosis)
19. X-ray of long bones (Gaucher disease, metastasis)
20. Flat plate of abdomen for spleen size (splenomegaly)
21. Lymph node biopsy (Hodgkin disease)
22. Liver biopsy (cirrhosis)
23. Splenic aspirate (lymphoma, leukemia)
24. Splenoportogram and splenic pulp pressure (portal cirrhosis)
25. Purified protein derivative (PPD) test, intermediate, and skin tests for various fungi (see section on hemoptysis)
26. Skin biopsy (hemochromatosis)
27. Muscle biopsy (collagen disease, trichinosis)
28. Diagnostic ultrasound (cyst, splenic aneurysm)
29. CT scan (malignancy)
30. Liver—spleen scan (splenomegaly)
1.2 TECOMELLA UNDULATA

1.2.1 Introduction

The drug consists of heartwood, stem bark, leaves and seeds of *Tecomella undulata* (Sm.) Seem syn. *Tecomella undulata* (Roxb.) G. Don of famly bignoniaceae, commonly known as Rohida, is a well known plant in the Ayurvedic system of medicine.\(^5\)\(^-\)\(^7\) It is usually a shrub, found in small patches, but when cultivated it may grow as high as 12 meters with a girth up to 2.4 meters. The species has been identified as an important for environmental conservation in arid zones as a stabilizer of shifting sand dunes, providing shelter for wild life. It is also a very useful species for afforestation of the drier tracts due to its drought and fire resistant properties.\(^8\)\(^-\)\(^9\)

The bark of *Tecomella undulata* is strongly astringent and specified for diseases of liver and spleen, internal tumors and diseases of abdomen incl. ascitis. Charka prescribed powdered bark, its decoction and extract in clarified butter in jaundice, enlarged spleen, anemia, intestinal warms and urinary disorders. The paste of root was given in leucorrhoea.\(^10\)\(^-\)\(^11\)

The various chemical constituents isolated from the plant are radermachol, an unusual rare pigment and 2-isopropenynaphtho[2,3-\(b\)]furan-4,9-quinone along with lapachol, tecomaquinone-I, dehydro-\(\alpha\)-lapachone, \(\alpha\)-lapachone, \(\beta\)-lapachone, cluytyl ferulate, stigmasterol and \(\beta\)-sitosterol.

1.2.2 Habitat

Distribution of *Tecomella undulata* is restricted to the drier parts of the Arabia, southern Pakistan and northwest India up to an elevation of 1200 meters. In Pakistan it is found in Baluchistan and Sind. In India, it occurs naturally in Rajasthan, Punjab, Haryana, Gujarat and Maharashtra. It is also distributed in sub-himalyan tract from gonda (Uttar Pradesh), eastward to Bengal, Sikkim and Assam west, in western ghat and Andmans.\(^10\)
The species is mainly found to occur in western parts of Rajasthan such as Barmer, Jaisalmer, Jodhpur, Pali, Ajmer, Nagaur, Bikaner, Churu and Sikar districts. In other states its population is scanty and very rare.

1.2.3 General Characteristics

The trade name of the tree species is Desert teak or Marwar teak. It is nearly evergreen tree of arid and semi arid regions. It occurs on flat and undulating areas including gentle hill slopes and sometimes also in ravines. It is well adapted to drained loamy to sandy loam soil having pH 6.5-8.0. The species thrives very well on stabilized sand dunes, which experience extreme low and high temperatures. It grows in areas of scanty rainfall (annual 150-500mm) and high temperature (35°C to 48°C). It can withstand extreme low temperature (0°C to -2°C) during winter and high temperature (48°C to 50°C) in summers. The tree is a strong light demander. It is drought, frost, fire and wind hardy. At the time of flowering (December-February) it produces beautiful showy flowers in yellow, orange and red colors. Three types of flower bearing trees can be observed near to each other in the same vicinity.\textsuperscript{12-13}

It is rarely hardy and resistant to drought and used for forestation and landscaping of dry tracts. The tree is propagated from seeds and cuttings. Propagation is highly successful in well drained fibrous loam and it requires plenty of water in summer.\textsuperscript{14}

The wood is grayish or yellowish brown, close grained and mould with light streaks and is tough, strong and durable. It takes a fine polish and is reported to be highly prized for furniture, carving and agricultural implements. The heartwood contains a good amount of lopachol which is toxic and responsible for the fungus and termites resisting property of wood.\textsuperscript{14}

Bark of young plant is soft and greenish brown and it is hard and dark brown in tree. Its bark is up to 8 mm. thick in fully matured tree. The bark of young branches is employed for the treatment of syphilis and eczema. Preliminary investigations have shown that the bark possess mild relaxant, cardiotonic and cholestatic activity. It contains tecomin
(veratroyl-β-D-glucoside), C_{29} and C_{27} alkanes, C_{28} and C_{30} alkanols and β-sitosterols. It is used for tanning.\cite{14}

Leaves are 4-8 × 1-1.25 cm. narrowly oblong or linear oblong, simple, obtuse, margin undulate, membranous, dark green, glabrous above and petiole. Leave of this plant have heavy edges.

Flowers are pale yellow or deep orange-red, showy, large, 6.5-7.5 cm. long in corymbose racemes, arrange in few flowered from short lateral branches. The tree looks beautiful when in full bloom from March to April month.

Fruit is capsule, slightly curved and seeds of \textit{Tecomella undulata} are winged.\cite{15-18}

It has been reported that soil under tree \textit{Tecomella undulata} has appreciably higher concentration of various macro and micro nutrient elements.\cite{19}

\subsection{1.2.4 Phytochemical Investigations}

Extensive studies have been carried out on \textit{Tecomella undulata}. Various chemical constituents isolated from heartwood, bark, seed, leaf, flower and fruit have been presented in Table 1.1

\textbf{Table 1.1} \textbf{Chemical constituents isolated from heartwood, bark, seed, leaf, flower and fruit shell of \textit{T. undulata}.}

<table>
<thead>
<tr>
<th>S.N.</th>
<th>Constituents isolated</th>
<th>Parts</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Radermachol</td>
<td>Heartwood</td>
<td>20</td>
</tr>
<tr>
<td>2</td>
<td>2-Isopropenynaphtho[2,3-b]furan-4,9-quinone</td>
<td>Heartwood</td>
<td>20</td>
</tr>
<tr>
<td>3</td>
<td>Tecomaquinone-I</td>
<td>Heartwood</td>
<td>20</td>
</tr>
<tr>
<td>4</td>
<td>α-Lapachone</td>
<td>Heartwood</td>
<td>20</td>
</tr>
<tr>
<td>5</td>
<td>β-Lapachone</td>
<td>Heartwood</td>
<td>20</td>
</tr>
<tr>
<td>6</td>
<td>Cluyl ferulate</td>
<td>Heartwood</td>
<td>20</td>
</tr>
<tr>
<td>7</td>
<td>Undulatin</td>
<td>Heartwood</td>
<td>21</td>
</tr>
<tr>
<td>No.</td>
<td>Compound</td>
<td>Location</td>
<td>References</td>
</tr>
<tr>
<td>-----</td>
<td>-----------------------------------</td>
<td>---------------------------</td>
<td>------------</td>
</tr>
<tr>
<td>8</td>
<td>Tectoquinone</td>
<td>Heartwood</td>
<td>21</td>
</tr>
<tr>
<td>9</td>
<td>Octacosanyl ferulate</td>
<td>Heartwood</td>
<td>21</td>
</tr>
<tr>
<td>10</td>
<td>Deoxylapachol</td>
<td>Heartwood</td>
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</tr>
<tr>
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<td>Lopachole</td>
<td>Heartwood</td>
<td>14, 49</td>
</tr>
<tr>
<td>12</td>
<td>Tectol</td>
<td>Heartwood and Root</td>
<td>21, 22</td>
</tr>
<tr>
<td>13</td>
<td>Dehydro-α-lapachone</td>
<td>Heartwood and Root</td>
<td>20, 21, 22</td>
</tr>
<tr>
<td>14</td>
<td>Tecomin</td>
<td>Heartwood and Bark</td>
<td>21, 23, 26, 27, 28</td>
</tr>
<tr>
<td>15</td>
<td>Dehydroprotectol</td>
<td>Heartwood and Bark</td>
<td>21, 24</td>
</tr>
<tr>
<td>16</td>
<td>ξ-Sitosterol</td>
<td>Heartwood and Bark</td>
<td>25</td>
</tr>
<tr>
<td>17</td>
<td>Veratric acid</td>
<td>Heartwood and Bark</td>
<td>5</td>
</tr>
<tr>
<td>18</td>
<td>A wax alcohol ferulate</td>
<td>Heartwood and Bark</td>
<td>22</td>
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<tr>
<td>19</td>
<td>n-Triacontanol</td>
<td>Heartwood and Bark</td>
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</tr>
<tr>
<td>20</td>
<td>Tecomelloside</td>
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<tr>
<td>21</td>
<td>Stigmasterol</td>
<td>Heartwood, Bark and Leaves</td>
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<tr>
<td>22</td>
<td>Undulatoside A</td>
<td>Bark</td>
<td>29</td>
</tr>
<tr>
<td>23</td>
<td>Undulatoside B</td>
<td>Bark</td>
<td>21</td>
</tr>
<tr>
<td>24</td>
<td>Tecoside</td>
<td>Bark</td>
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<tr>
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<td>β-Sitosteryl acetate</td>
<td>Bark</td>
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<tr>
<td>26</td>
<td>p-Hydroxybenzoic acid</td>
<td>Bark</td>
<td>31</td>
</tr>
<tr>
<td>27</td>
<td>β-Amyrin</td>
<td>Bark and leaves</td>
<td>31</td>
</tr>
<tr>
<td>28</td>
<td>Campesterol</td>
<td>Bark and leaves</td>
<td>31</td>
</tr>
<tr>
<td>29</td>
<td>Alkanols</td>
<td>Bark and leaves</td>
<td>5</td>
</tr>
<tr>
<td>30</td>
<td>Alphanamixininin</td>
<td>Bark and fruit shell</td>
<td>10</td>
</tr>
<tr>
<td>31</td>
<td>β-Sitosterol</td>
<td>Bark, leaves &amp; flowers</td>
<td>5, 14, 20, 32</td>
</tr>
<tr>
<td>32</td>
<td>Rutin</td>
<td>Flowers</td>
<td>32</td>
</tr>
</tbody>
</table>
1.2.5 Pharmacological Investigation
In recent years many researchers have examined the effect of *Tecomella undulata* used traditionally by indigenous healers and herbalists to support function of various body
parts and treat diseases in human and animals. In most cases, research has confirmed traditional experience and wisdom by discovering the mechanisms and modes of action as well as reaffirming the therapeutic effectiveness of plant or plant extracts in clinical studies.

1.2.5.1 **Anti microbial activity**

Biochemical analysis indicated that *T. undulata* leaves have oleanolic acid, ursolic acid and betulinic acid, compounds that are strong HIV inhibitors. Octadimethyl succinate derivatives of oleanolic acid and betulinic acid have been reported to be 24 times more active than AZT, a drug that is currently used for checking the spread of AIDS.\(^{36}\)

Other compounds isolated from the leaves of *T. undulata* are \(\beta\)-sitosterol, triacontanol, cirsimaritin, cirilineol, pentatetracontanol and 4,5-dihydroxy-3,6,8-trimethoxy flavone. Both aqueous and alcoholic leaf and stem extracts of *T. undulata* showed growth inhibition of *Salmonella typhi*, a causal organism of typhoid fever.

Sumitra Chandra et al. works on antibacterial activity with methanolic and aqueous extracts of *Tecomella undulata*. They found that plant extracts were more active against Gram-positive bacteria than against Gram-negative bacteria. The most susceptible bacteria were *B. subtilis*, followed by *S. epidermidis*, while the most resistant bacteria were *P. vulgaris*, followed by *S. typhimurium*. The antibacterial activity of aqueous and methanol extracts was determined by agar disk diffusion and agar well diffusion method. The methanol extracts were more active than the aqueous extract.\(^{37-38}\)

1.2.5.2 **Central analgesic activity**

*Tecomella undulata* has significant analgesic activity. Whole plant of *Tecomella undulata* was extracted with absolute methanol by Ahmad F. et al. using the hot water tail immersion test in mice and carrageenan induced pedal edema in rats, both extracts were tested for oral analgesic potential. Result showed that *T. Undulata* had analgesic potential when compared with aspirin.\(^{39}\) This extract probably act on opioidergic receptors and appear to be promising analgesic agent. However, further experiments will possibly
define this pharmacological effect. If confirmed it, may become of importance for human clinical treatments.40-41

1.2.5.3 Hepatoprotective activity

Stem bark of *Tecomella undulata* have strong activity against thioacetamide induced hepatotoxicity. Oral administration of *Tecomella undulata* at 1000 mg/kg resulted in a significant reduction in serum aspartate aminotransaminase (35% and 31%, respectively), alanine aminotransaminase (50% and 42%, respectively), gamma glutamyl transpeptidase (56% and 49%, respectively), alkaline phosphatase (46% and 37%, respectively), total bilirubin (61% and 48%, respectively) and liver MDA levels (65% and 50%, respectively), and significant improvement in liver glutathione (73% and 68%, respectively) when compared with thioacetamide damaged rats. Histology of the liver sections of the animals treated with the extracts also showed dose-dependent reduction of necrosis.42-43

1.2.5.4 Splenomegalic activity

Rohida bark has their significant action in splenomegaly. Specific action how it works in spleen is not known but it used in ayurveda for treatment of enlargement of spleen.44-46

1.2.5.5 Immune modulator activity

*Tecomella undulata* possesses diverse biological activities and having bio- modulatory and immunomodulatory functions. Its alchohalic extract influence immune system viz. increase phagocytic activity of macrophages, stimulating the production of antibodies and cytokines, increase accumulation of NK cells into tissue and activation and mobilization of T and B cells.42,47

*Tecomella undulata* with a herbal combination of *Moringa oleifera, Boerhavia diffusa, Onosma bracteatum, Bauhinia variegata, Sphaneanthus indicus, Chlorophytum borivilianum, Ficus racemosa, and Cyperus rotundus* is effective for the treatment a wide range of physiological and pathological conditions in the human body resulting from a
weakened or deteriorating immune system. This combination herbal preparation has been found to be particularly useful in maintaining the normal physiological functions of the immune system, in regulating the immunological functions and all the aberrations that occur due to the subtle immunological imbalances and reduced immunity, and to restore and improve the immune function in individuals exhibiting a weakened or deteriorating immune response.

It have beneficial effects and to improve the quality of life in individuals experiencing all types of cancer, especially those that directly weaken the immune system, in individuals affected with HIV and AIDS, in individuals exhibiting failing immunity due to old age, and all other conditions of the human body that negatively affect the immune system through the following mechanisms: (1) by stimulating the production of growth factors responsible for production of the cells of the immune apparatus, like lymphocytes, macrophages, Langerhans cells, histiocytes, etc.; (2) by enhancing the immune response due to the production of new cells and replacing the aging and functionally incompetent cells of the immune system; (3) by mopping up the free radicals generated by the metabolism of cancer cells, the anti-retroviral metabolism in cells of individuals affected with HIV or AIDS, and during the aging process (i.e. antioxidant effect); and (4) by stimulating the immune apparatus to produce antibodies and to form immune complexes (i.e. immunostimulatory effect).

It can also be used as a chemo protective or radio protective agent in individuals affected with cancer, wherein it can be used as an adjuvant to conventional treatments, such as chemotherapy and radiotherapy, to reduce the adverse side effects of these therapies. The combination herbal preparation also exhibits radio sensitizing and chemo sensitizing effects in cancer patients by enabling the tumor to become more sensitive to the effects of these two standard modalities of conventional anticancer therapy. Improved sensitivity of the tumor to radiotherapy and chemotherapy also helps in effectively reducing the required dosage of these therapies in order to achieve the prescribed therapeutic effects, thereby reducing and alleviating the powerful and devastating adverse toxic effects exerted by radiotherapy and chemotherapy in cancer treatment.48
1.2.5.6 **Tonic for animals**

*Tecomella undulata* used for recumbent the animal. The bark of the tree is ground to a powder and 100g of the same is administered daily till the animal recovers.\(^\text{47}\)

1.2.5.7 **Vermifuge activity**

Lopachol is present in heartwood of *Tecomella undulata*, which is toxic in nature and have fungus and termites resistant property.\(^\text{14, 49}\)

1.2.5.8 **Anti infective activity (Skin diseases)**

*Tecomella undulata* is used to cure the parasitic skin diseases and used for poulticing in cutaneous diseases. Bark and wood is soaked in water for two days. This is then distilled and the distillate collected is applied on the eczema. The bark of the young branches is often employed in Sind as remedy for syphilis.\(^\text{47, 50}\)
1.3 PHYTOSOMES

Over the past century, phytochemical and phytopharmacological sciences established the compositions, biological activities and health promoting benefits of many plant products. Most of the biologically active constituents of plants are polar or water soluble molecules. However, water soluble phytoconstituents (like glycosides, tannins, terpenoids, etc.) are poorly absorbed either due to their large molecular size which cannot absorb by passive diffusion, or due to their poor lipid solubility; severely limiting their ability to pass across the lipid-rich biological membranes, resulting poor bioavailability. It has often been observed that the isolation and purification of an extract may lead to a partial or total loss of specific activity for the natural constituent synergy becomes lost. Very often the chemical complexity of the crude or partially purified extract seems to be essential for the bioavailability of the active constituents. Extracts when taken orally some constituents may be destroyed in the gastric environment. As standardized extracts are established, poor bioavailability often limits their clinical utility due to above said reasons. It has been observed that complexation with certain other clinically useful nutrients substantially improves the bioavailability of such extracts and their individual constituents. Over the past several years, great advances have been made on development of novel drug delivery systems (NDDS) for plant actives and extracts. The variety of novel herbal formulations like polymeric nanoparticles, nanocapsules, liposomes, phytosomes, nanoemulsions, microsphere, transfersomes, and ethosomes has been reported using bioactive and plant extracts. The novel formulations are reported to have remarkable advantages over conventional formulations of plant actives and extracts which include enhancement of solubility, bioavailability, protection from toxicity, enhancement of pharmacological activity, enhancement of stability, improved tissue macrophages distribution, sustained delivery, and protection from physical and chemical degradation. The nutrients so helpful for enhancing the absorption are the phospholipids. Phytosome is a patented technology developed by a leading manufacturer of drugs and nutraceuticals, to incorporate standardized plant extracts or water soluble phytoconstituents into phospholipids to produce lipid compatible molecular complexes, called as phytosomes and so vastly
improve their absorption and bioavailability. In liposomes no chemical bond is formed; the phosphatidylcholine molecules surround the water soluble substance. There may be hundreds or even thousands of phosphatidylcholine molecules surrounding the water soluble compound. In contrast, with the phytosome process the phosphatidylcholine and the plant components actually form a 1:1 or a 2:1 molecular complex depending on the substance(s) complexed, involving chemical bonds (Figure 1.3). Phospholipids are complex molecules that are used in all known life forms to make cell membranes. In humans and other higher animals the phospholipids are also employed as natural digestive aids and as carriers for both fat-miscible and water miscible nutrients. They are miscible both in water and in lipid environments, and are well absorbed orally. Phytosomes are more bioavailable as compared to conventional herbal extracts owing to their enhanced capacity to cross the lipoidal biomembrane and the systemic circulation. (Figure 1.4) The phytosome process has been applied to many popular herbal extracts including Ginkgo biloba, grape seed, hawthorn, olive fruits and leaves, milk thistle, green tea, ginseng, kushenin, marsupsin and curcumin. Increased bioavailability of the phytosomes over the simpler, non-complexed plant extract has been demonstrated by pharmacokinetics and activity studies, conducted in animals as well as human beings. These compounds can be considered novel entities on the basis of their physiochemical and spectroscopic characteristics. Presently phytosomes are used primarily in cosmetics to deliver water soluble substances to the skin. This technology is also useful in pharmaceutical formulations intended for treatment of oral cavity in which the contact times are very short because phospholipid allows a greater adhesion of the product itself to the surfaces it comes into contact with.52-54

1.3.1 Advantages of Phytosomes
1. Marked enhancement of bioavailability.
2. Phytosome process produces a little cell whereby the valuable components of the herbal extracts are protected from destruction by digestive secretions and gut bacteria.
3. Assured delivery to the tissues.
Figure 1.3: Cross section of Phytosome showing major difference between Phytosome and Liposome

Figure 1.4: Cross section showing attachment of lipoidal biomembrane and Phytosome delivery form
4. No compromise of nutrient safety.
5. Dose requirement is reduced due to absorption of chief constituent.
6. Entrapment efficiency is high and more over predetermined because drug itself in conjugation with lipids is forming vesicles.
7. No problem of drug entrapment.
8. Phytosomes shows better stability profile because chemical bonds are formed between phosphatidylcholine molecules and phytoconstituent.
9. Phosphatidylcholine used in the phytosome process besides acting as a carrier also nourishes the skin, because it is essential part of cell membrane.
10. Phytosomes are also superior to liposomes in skin care products.
11. Significantly greater clinical benefit.
12. The particular structure of phytosome elicits peculiar properties and advantages in cosmetic application.

1.3.2 Physical and Chemical Properties of Phytosomes

They are lipophilic substances with a definite melting point, freely soluble in nonpolar and aprotic solvents in which the hydrophilic moiety is not present. They are moderately soluble in fats and insoluble in water. When treated with water, they assume a micelle shape, forming structures which resemble liposome. In these complexes, the polar head of the phospholipid is involved while the fatty acid moieties retain a high degree of mobility conferring marked lipophilia at the new molecule. In the 1H-NMR spectrum, the signals of the complexed substances undergo a strong broadening so as they can no more be evidenced in the spectra. In the 13C-NMR spectrum, the signals of the complexed substances as well as those of the choline and glycerin portion of the phospholipid can no more be recorded. The phosphorous nucleus itself undergoes a band broadening which indicates that it is involved in complex formation. In both the 1H-NMR and 13C-NMR spectra, only the lipid chain signal appear even showing some immobilization. The kind of signals proves the interaction between polar head and active sites of the complex where the lipid chains are not involved since they are free to rotate and give complex its lipophilic character. 55-58
1.3.3 Method of Preparation

Phytosomes are prepared by reacting natural or synthetic phospholipids with active components like bioflavonoid, flavolignan and polyphenolic constituents. Solvent Evaporation method is the most common technique used for the preparation of phytosomes. Phytosomes of ginsenoside, puerarin and kushenin are prepared in this manner. Mechanical Dispersion method is used for the preparation of marsupisin-phospholipid complexes.

Phospholipids is dissolved in a suitable solvent and active ingredient is added drop by drop while sonicating the solution. Phospholipid complex is sometimes prepared under reflux and stirring conditions to effect complete interaction. Curcumin phospholipids complexes are prepared by adding the phospholipids to the ethanol solution of the hydroalcoholic extract of turmeric rhizomes, under reflux and with stirring. Prepared complex called phytosome can be isolated by precipitation with nonsolvent, lyophilization, spray drying or vacuum drying.59-68

1.3.4 Difference between Phytosomes and Liposomes

Fundamental differences exist between a Phytosome and a liposome. In liposomes, the active principles are water soluble and are hosted in the inner cavity, with little, if any, interaction taking place between the hydrophilic principle and the surrounding lipid core. Conversely, Phytosomes host their polyphenolic guest, generally little soluble both in water and in lipids, at their surface (Figure 1.3), where the polar functionalities of the lipophilic guest interact via hydrogen bonds and polar interactions with the charged phosphate head of phospholipids, forming a unique arrangement that can be evidenced by spectroscopy.

The Phytosome formulation also increases the absorption of active ingredients when topically applied on the skin, and improves systemic bioavailability when administered orally. In water medium, a Phytosome will assume a micellar shape, forming a spherical structure, overall similar to a liposome, but with a different guest localization.
Table 1.2:  Difference between Phytosome and Liposome

<table>
<thead>
<tr>
<th>Phytoosomes</th>
<th>Liposomes</th>
</tr>
</thead>
<tbody>
<tr>
<td>In phytosomes active chemical constituents molecules are anchored through chemical bonds to the polar head of the phospholipids.</td>
<td>In liposomes, the active principle is dissolved in the medium of the cavity or in the layers of the membrane. No chemical bonds are formed.</td>
</tr>
<tr>
<td>In phytosomes, phosphatidylcholine and the individual plant compound form a 1:1or 2:1complex depending on the substance.</td>
<td>In liposomes, hundreds and thousands of phosphatidylcholine molecules surround the water soluble molecule.</td>
</tr>
</tbody>
</table>

Table 1.3:  Herbal drugs and their Phytosomes

<table>
<thead>
<tr>
<th>S. N</th>
<th>Herbal Drug</th>
<th>Phytosome</th>
<th>Phytoconstituents complexed with phosphatidylcholine</th>
<th>Indications</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Ginkgobiloba</td>
<td>Ginkgocelec Phytosome</td>
<td>(a) Dimeric flavonoids (^{69}) (b) terpenoids (gikgolides and bilobalide) (^{70-71})</td>
<td>(a) Vasoactive agent (b) Anti-inflammatory agents</td>
</tr>
<tr>
<td>2</td>
<td>Silybum Marianum</td>
<td>Silybin Phytosome</td>
<td>(a) Flavolignans (^{72-73}) (Silybin) (b) Flavanolignans (^{74}) (Silymarin)</td>
<td>(a) Antioxidant and hepatoprotective (b) Anti-inflammatory Anti-aging</td>
</tr>
<tr>
<td>3</td>
<td>Crataegus Oxyacantha</td>
<td>Hawthome Phytosomes</td>
<td>Flavonoids (^{75-78})</td>
<td>Antioxidant, cardioprotective, Food product</td>
</tr>
<tr>
<td>4</td>
<td>Camellia Sinensis</td>
<td>Greenselect® Phytosome</td>
<td>Catechins and their gallate derivatives (^{79})</td>
<td>Antioxidant, cardioprotective, food product</td>
</tr>
<tr>
<td>5</td>
<td>Panax Ginseng</td>
<td>Ginselect\textsuperscript{TM}</td>
<td>Saponins (^{80-82})</td>
<td>Anti-aging</td>
</tr>
<tr>
<td></td>
<td><strong>Terminalia Serica</strong></td>
<td>Sericosides Phytosome</td>
<td>Sericosides&lt;sup&gt;83&lt;/sup&gt;</td>
<td>Skin restructuring, capillary protecting, wound healing, anti-oedema, anti-inflammatory.</td>
</tr>
<tr>
<td>---</td>
<td>-------------------------</td>
<td>----------------------</td>
<td>---------------------------</td>
<td>--------------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>7</td>
<td><strong>Vaccinium Myrtillus</strong></td>
<td>Mirtoselect® Phytosome</td>
<td>Antcinode&lt;sup&gt;84&lt;/sup&gt;</td>
<td>Antioxidant</td>
</tr>
<tr>
<td>8</td>
<td><strong>Vitis vinifera</strong></td>
<td>Leucoselect® Phytosome</td>
<td>Monomeric flavan-3-ols (catechins and epicatechins &amp; their gallate derivatives) together with their polymer procyanidins&lt;sup&gt;85,86&lt;/sup&gt;</td>
<td>Cardiovascular protectant, anti-inflammatory, antioxidant.</td>
</tr>
<tr>
<td>9</td>
<td><strong>Serenoa repens(Bartr)</strong></td>
<td>Sabalselect® Phytosome</td>
<td>Phytosterols</td>
<td>Non-cancerous prostate enlargement.</td>
</tr>
<tr>
<td>10</td>
<td><strong>Melilotus Officinalis</strong></td>
<td>Lymphaselect™ Phytosome</td>
<td>Coumarin</td>
<td>Effective in the symptomatic treatment of chronic venous insufficiency, varicosities, hemorrhoids, thrombophlebitis, post-surgical edema formation, and of severe lymphatic disorders such as lymphedema.</td>
</tr>
<tr>
<td>11</td>
<td><strong>Olea Europaea</strong></td>
<td>Oleaselect™ Phytosome</td>
<td>Polyphenols</td>
<td>Antioxidant, inhibit harmful oxidation of LDL cholesterol, anti-inflammatory.</td>
</tr>
<tr>
<td>12</td>
<td><strong>Echinacea angustifolia</strong></td>
<td>Polinacea™</td>
<td>Echinacosides, inulin&lt;sup&gt;87&lt;/sup&gt;</td>
<td>enhances immune function in response to a toxic challenge</td>
</tr>
<tr>
<td>13</td>
<td><strong>Curcuma Longa Linn</strong></td>
<td>Curcumin phytosome</td>
<td>Curcumin (polyphenols)&lt;sup&gt;88&lt;/sup&gt;</td>
<td>Cancer chemopreventive agent.</td>
</tr>
</tbody>
</table>
1.3.5 Evaluation of Phytosomes

Various spectroscopic and in-vitro and in-vivo evaluations are applied on phytosomes. These complexes can be characterized by Transmission Electron Microscopy (TEM), $^1$H-NMR, $^{13}$C-NMR, $^{31}$P-NMR and FT-IR. Models of in-vitro and in-vivo evaluations are selected on the basis of expected therapeutic activity of biologically active phytoconstituents present in phytosomes. Complexation increases the activity of the active principle. A chemical spectral characteristic is determined in phospholipids complexes using IR and UV spectroscopic study. Liquid chromatography/atmospheric pressure chemical ionization mass spectrometry (LC/APCI-ITMS) proved to be a very powerful tool for pharmacokinetic studies of phytochemicals. This technique is applied to evaluate the levels of ginkgolides A and B and bilobalide in plasma of ginkgolides A and B and bilobalide in plasma of volunteers after administration of Ginkgo biloba extracts in free (Ginkgoselect) or phospholipid complex (Ginkgoselect Phytosome) forms. The effects of Ginkgo biloba dimeric flavonoids in Phytosome form on the vasomotor activity and skin microcirculation of the cheeks, hands, limbs and female breast are studied in human subjects by Infrared-Photo-Pulse-Plethysmography, Laser Doppler Flowmetry, High Performance Contact Thermography, Computerized Videothermography, and Optic Probe Video capillaroscopy. In-vivo studies are performed on Beagle dogs, rodents, wistar rats to compare pharmacokinetics parameters between pure extracts and its phospholipid complex.\textsuperscript{89,90}

1.3.6 Advances in Phytosome technology (Pre-clinical and Clinical trials)

1. In a very recent study the tissue and blood effects of high-dose silybin-phytosome in prostate cancer patients was determined. Patients received silybin-phytosome for 14-31 days (mean was 20 days) prior to surgery. Silibinin blood levels were measured 1 h after the first silybin-phytosome dose with a mean value of 19.7 µM. One of the treated patients developed a grade 4 post-operative thromboembolic event. The other observed toxicities in the treatment group were mild: four subjects had diarrhea and one had asymptomatic grade 2 hyperbilirubinemia which was transient. The results indicate that high-dose oral silybin-phytosome achieves high
blood concentrations transiently, but low levels of silibinin are seen in prostate tissue.\textsuperscript{91}

2. Green Phytosome were prepared and studied in 100 obese subjects (both male and female, divided into 2 groups of 50 each). Group 1 was given hypocaloric diet with green tea phytosome. Group 2 was given only hypocaloric diet. After 90 days, parameters like weight, body mass index, low density lipid, High density lipid, total cholestrol, triglycerides, insulin, growth factor, cortisol were determined. All parameters were improved in both groups but there was more weight loss in green tea phytosome group than in diet only group (14 kg loss versus 5 kg loss). Also, no adverse effects were reported during and after trial.\textsuperscript{92}

3. Another method currently being investigated is complexing curcumin with a phospholipid, known as a phytosome. The phosphatidylcholine curcumin complex (Meriva\textregistered) is more readily incorporated into lipophilic cell membranes, making it significantly more bioavailable than unbound curcumin. In rats, peak plasma concentration and AUC were five times higher for Meriva than for unbound curcumin.\textsuperscript{93}

4. Phytosomes of curcumin (flavonoid from turmeric, Curcuma longa) and naringenin (flavonoid from grape fruit, vitis vinifera) showed higher antioxidant activity than pure curcumin in all dose levels tested.\textsuperscript{94} In a study the bioavailability of silybin in rats was found to increase remarkably after oral administration of prepared silybinphospholipid complex Phytosome due to an impressive improvement of the lipophilic property of silybin-phospholipid complex and improvement of the biological effect of silybin.\textsuperscript{95}

5. \textit{Ginkgo biloba} phytosome treatment was found to increase superoxide dismutase, catalase, glutathione peroxidase and glutathione reductase activities in all the brain regions compared with those treated only with sodium nitrite. \textit{Ginkgo biloba} phytosomes were administered to Wistar rats at 50 mg/kg and 100 mg/kg for 7 and 14 days. Chemical hypoxia was induced by administration of sodium nitrite (75 mg/kg) 1 h after the last administration of treatment. Thirty minutes after sodium
nitrite administration, the animals were killed and the cerebral cortex, cerebellum, hippocampus and striatum were isolated and homogenized. The supernatants were used for the estimation of the antioxidant enzymes superoxide dismutase, catalase, glutathione peroxidase and glutathione reductase.\textsuperscript{96}

6. \textit{Herba Epimedii} flavonoid phytosomes (EFP) were prepared by means of solvent evaporation technique and the accumulative dissolution of different ratios of EFP-PVP precipitates was investigated by means of dissolution release. For optimized preparation solvent-tetrahydrofuran, lecithin to PVP 2.5 times, temperature 40°C and reaction 3 h. Oil/water apparent partition coefficient of icariin was enhanced more than 4 times by phospholipid. The accumulative dissolution of \textit{Herba Epimedii} flavonoids of EFP-PVP precipitate was significantly higher than that of its physical mixture and \textit{Herba Epimedii} extract tablet.\textsuperscript{97}

7. Patients suffering from chronic hepatitis (viral, alcohol or drug induced) treated with silybin phytosome at a dose of 120 mg either twice daily or thrice daily for up to 120 days, liver function returned to normal faster in patients taking silybin phytosome compared to a group of controls (49 treated with commercially available silymarin, 117 untreated or given placebo).\textsuperscript{98}

8. In a study silymarin (a standardized mixture of flavanolignans extracted from the fruits of \textit{S. marianum}) phytosomes showed much higher specific activity and a longer lasting action than the single constituents, to percent reduction of odema and inhibition of myeloperoxidase activity.\textsuperscript{99}

9. A human study was conducted to design the absorption profile of silybin when directly bound to phosphatidylcholine. Plasma levels of silybin were determined after administration of single oral doses of silybin phytosome and a similar amount of silybin from milk thistle in healthy volunteers. The results indicated that the absorption of silybin from silybin phytosome was approximately seven times greater compared to the absorption of silybin from regular milk thistle extract.\textsuperscript{100}
1.4 REVIEW OF LITERATURE

1. **Alvala R, Mallika A. et al. (2011)** established a scientific validation for the antitumor effects of Tecomella undulata bark and explored the mechanistic pathway in chronic myeloid leukemia cell line, K562. The investigation clearly demonstrated the potential antitumor effect of CTUB, thereby validating the traditional claim.\(^\text{101}\)

2. **Goyal R, Sharma PL et al. (2010)** studied the pharmacological effect of *Tecomella undulate*: bark against carrageenan induced paw edema and cotton pellet induced granuloma in rat. Chronic inflammation was induced by cotton pellet induced granuloma method. Serum nitrate/nitrate estimation was also done as an index of inflammatory reactions. Acute toxicity study was also done using Swiss albino mice. Butanolic and water fractions of *Tecomella undulate* (200 & 400 mg/kg) and indomethacin (10 mg/kg) were used as test drugs. Carrageenan caused a marked increase in rat paw volume due to edema formation. *T. undulate*: butanolic fraction significantly inhibited paw volume in successive hours similar to indomethacin. Interscapular implanted cotton caused significant increase in granuloma wt. and serum nitrate/nitrite level in control group. However, the test drugs lowered the effects of cotton pellet induced chronic inflammation. Therefore, the results may conclude that the bark of *T. undulate* is having a pharmacological potential to treat acute and chronic inflammation in rat.\(^\text{102}\)

3. **Khatri A, Garg A et al. (2009)** Evaluate the hepatoprotective activity of aerial parts of stem bark of *Tecomella undulata* against thioacetamide-induced hepatotoxicity. Oral administration of *Tecomella undulata* at 1000 mg/kg resulted in a significant reduction in serum aspartate aminotransaminase (35% and 31%, respectively), alanine aminotransaminase (50% and 42%, respectively), gamma glutamyl transpeptidase (56% and 49%, respectively), alkaline phosphatase (46% and 37%, respectively), total bilirubin (61% and 48%, respectively) and liver MDA levels
(65% and 50%, respectively), and significant improvement in liver glutathione (73% and 68%, respectively) when compared with thioacetamide damaged rats.\textsuperscript{103}

4. **Rana MG, Katbamna RV (2008)** studied the hepatoprotective effect of crude Methanolic extract from the bark parts of *Tecomella undulata*. The methanolic extract obtained from bark parts of *Tecomella undulata* was evaluated for hepatoprotective activity in rats by inducing liver damage by carbon tetrachloride.\textsuperscript{104}

5. **Hussain F, Badshah L et al. (2006)** studied folk medicinal uses of some plants of South Waziristan, Pakistan. They prepared small pills weighing up to 0.5 gm are made from the powdered bark with rausugar (gur). Two or three pills were taken daily in the morning for curing various liver, lung and chest problems.\textsuperscript{105}

6. **Jigna P, Darshana J et al. (2005)** worked on plant extract of *T. undulata*. Results showed that plant extract was more active against Gram-positive bacteria than against Gram-negative bacteria. The most susceptible bacteria were B. subtilis, followed by S. epidermidis, while the most resistant bacteria were P. vulgaris, followed by S. typhimurium.\textsuperscript{106}

7. **Vishwakarma RK (2000)** worked on Toxic effect of various plant part extracts on organism (S. Typhe) of typhoid fever.\textsuperscript{107}

8. **Gehlot D, Bohra A (2000)** worked on stem extract of *Tecomella undulata* and find that extract having antibacterial activity against S. typhpe.\textsuperscript{108}

9. **Ahmad F, Khan RA et al. (1994)** worked on *Tecomella undulata* extract absolute methanol. Using the hot water tail immersion test in mice and carrageenan induced pedal edema in rats, extract was tested for their oral analgesic activity. Scientists find T. undulata had analgesic potential when compared with aspirin.\textsuperscript{109}
10. **Naik SR, Panda VS (2008)** tested the protective effects of Ginkgoselect Phytosome® (GBP) on Rifampicin (RMP) induced hepatotoxicity and the probable mechanism(s) involved in this protection were investigated in rats. Liver damage was induced in Wistar rats by administering rifampicin (500 mg/kg, p.o.) daily for 30 days. Simultaneously, GBP at 25 mg/kg and 50 mg/kg, and the reference drug silymarin (100 mg/kg) were administered orally for 30 days/daily to RMP treated rats. Levels of marker enzymes (SGOT, SGPT and SALP), albaumin (Alb) and total proteins (TP) were assessed in serum. The effects of GBP on lipid peroxidation (LPO), reduced glutathione (GSH), superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPX) and glutathione reductase (GR) were assayed in liver homogenates to evaluate antioxidant activity.\(^\text{110}\)

11. **Panda VS, Naik SR (2008)** were investigated the protective effects of Ginkgobiloba Phytosomes (GBP) in isoproterenol (ISO)-induced cardiotoxicity and the antioxidant activity involved in this protection in rats. Myocardial infarction was produced in rats with 65, 85, 120 and 200mg/kg of ISO administered subcutaneously (sc) twice at an interval of 24 h. An ISO dose of 85mg/kg was selected for the present study as this dose offered signcant alteration in biochemical parameters and moderate necrosis in heart. Effect of GBP oral treatment for 21 days at two doses (100mg and 200mg/kg body weight) was evaluated against ISO (85mg/kg, sc)-induced cardiac necrosis. Levels of marker enzymes (AST, LDH and CPK) were assessed in serum and heart, antioxidant parameters viz., reduced glutathione (GSH), superoxide dismutase(SOD), catalase (CAT), glutathione peroxidase (GPx) and glutathione reductase (GR) and malondialdehyde (MDA) were assayed in heart homogenate. Significant myocardial necrosis, depletion of endogenous antioxidants and increase in serum levels of marker enzymes were observed in ISO-treated animals when compared with the normal animals. GBP elicited a significant cardioprotective activity by lowering the levels of serum marker enzymes and lipid peroxidation and elevated the levels of GSH, SOD, CAT, GPx and GR.\(^\text{111}\)
12. **Ulrich M, Sanja C et al. (2008)** used a “dual asymmetric centrifuge (DAC)” technique for preparing liposomes. DAC differs from conventional centrifugation by an additional rotation of the sample around its own vertical axis: While the conventional centrifugation constantly pushes the sample material outwards, this additional rotation constantly forces the sample material towards the center of the centrifuge. This unique combination of two contra rotating movements results in shear forces and thus, in efficient homogenization.\(^\text{112}\)

13. **Nobuhiro Y, Yuko Y al. (2007)** developed a novel lipid analog based on amino acids for liposome modification. It consisted of three different kinds of amino acid derivatives and two fatty acids, and can react directly with the peptide synthesized first on resin by Fmoc solid-phase synthesis. In this study, lipid analog conjugated with HIV-TAT peptide (domain of human immunodeficiency virus TAT protein) was synthesized and successfully incorporated into liposome. The liposome containing the lipopeptide bearing HIV-TAT exhibited efficient cellular uptake.\(^\text{113}\)

14. **Pierluigi M, Antonella DP et al. (2006)** Liquid chromatography/atmospheric pressure chemical ionization ion trap mass spectrometry (LC/APCI-ITMS) was applied to determine the concentration of terpene lactone in plasma of guinea pigs after chronic administration of *Ginkgo biloba* extract enriched in ginkgoterpenes in free form (IDN 5380) or complexed with soy phospholipids (IDN 5381). Oral treatment of the animals with ginkgoterpenes resulted to inhibit the bronchoconstriction (ITP) and the concomitant increase of the levels of thromboxane B2 (TXB2) in the circulation caused by histamine (HIST) and platelet activating factor (PAF) in normal guinea pigs or by ovalbumin (OA) in actively sensitized guinea pigs\(^\text{114}\)

15. **Jonathan P, Huiming Y et al. (2003)** evaluated the effect of liposome delivery on the controlled release and therapeutic efficacy of ciprofloxacin against intracellular *Francisella tularensis* infection in vivo in this study. Ciprofloxacin was encapsulated
in small unilamellar vesicles by a remote loading procedure using an ammonium sulfate gradient. This procedure produced uniform sized liposomes (100 nm). Following administration of liposome encapsulated ciprofloxacin by intravenous injection or aerosol inhalation, levels of ciprofloxacin in sera, lungs, liver and spleen were determined using $^{14}\text{C}$-ciprofloxacin as radiotracer for ciprofloxacin.  

16. **Kasprzak KS (1985)** examined the nature of the different effects of magnesium and calcium on the uptake and distribution of carcinogenic doses of cadmium in rats. The rats received a single sc. injection of cadmium chloride (0.02 mmol/kg or 0.04 mmol/kg) and sc injections (1 daily) of 0.16 mmol/kg calcium acetate (0.16 mmol/kg), or 4 mmol/kg magnesium acetate, or saline on the day before, the day of and the day after the cadmium chloride dosing. The conc. of cadmium in tissues was detected on the 4th, the 15th, and the 45th day after the cadmium chloride injection. The concn of cadmium in tissues on day 4 was ranked as follows: liver > kidney > the injection site skin > pancreas > spleen > heart > lung > distant skin > testes > blood.  

17. **Henke et al. (1970)** worked on mammals and investigated that cadmium is virtually absent at birth but will accumulate with time, especially in liver and kidneys. The primar period of rapid renal concentration may occur during the early years of life and 50-75% of the total body burden will be found in these two organs.  

18. **Nomiyama et al. (1978)** reported that absorption of cadmium from the gastrointestinal tract depends on species, type of cadmium compound, dose size and frequency, age and interaction with various dietary components.  

19. **Decker et al. (1958)** proved that, when cadmium chloride was given to rats in drinking water over a period of 122 months, less than 1% of the total dose ingested was retained in liver and kidney.
20. **Gunn & Gould, (1957) and Nordberg & Nishiyama, (1972) studied that in** various species, after administration of a single dose *per os* or parenterally, the highest burden initially occurs in the liver but kidney levels may increase over several months to exceed liver level. Pancreas and spleen also acquire relatively high concentrations. The accumulation of cadmium in the liver and subsequent redistribution to the kidney is probably due to efficient metallothionein synthesis in the liver; cadmium-metallothionein may be slowly released into the plasma, filtered through the glomeruli and reabsorbed in the tubules as previously mentioned. Thus in high exposure situations, even after discontinuation of exposure, concentrations of cadmium in the renal cortex may be maintained for a prolonged period or may even continue to increase if liver stores are high.\(^{120-121}\)

21. **Friberg et al. (1974, 1975, 1986) and Nomiyama (1978) has been reviewed the fate of cadmium after chronic exposure by various route has been reviewed by. Initially, cadmium in liver increases rapidly and is redistributed slowly to the kidney so that the higher the intensity of exposure, the higher the ratio of liver to kidney concentrations. The route of administration can also affect this ratio.\(^{122-125}\)**

22. **Tarasenko et al. (1974) Vorobjeva & Sabalina (1975) worked on LD\(_{50}\) of various cadmium salts. Oral administration of single high doses of cadmium compounds causes desquamation of the epithelium, necrotic changes in the gastrointestinal mucosa and dystrophic changes in heart, liver and kidneys.\(^{126-127}\)**
1.5 AIM OF WORK

The spleen is an important organ in the immune system. The functions of the spleen normally include clearance of invading organisms (bacteria) in the blood from the circulation, production of antibodies for the immune system, and removal of abnormal blood cells.

Various types of infections, diseases affecting blood cells, increased splenic blood flow, infiltration of the spleen from other diseases, inflammatory conditions and diseases invading the spleen are some common reasons for the spleen to enlarge.

In many cases the spleen can enlarge by performing its normal functions in response to another medical condition. When drug given to the patient for treatment of another medical condition then many time spleen sizes may not reduced to normal size and many symptoms associated with enlarged spleen are related to the underlying cause of the enlargement. These may include fever, night sweats, paleness (pallor), generalized weakness, fatigue, easy bruising, and weight loss.

Liquid solubility and molecular size are the major limiting factors for molecules to pass the biological membrane to be absorbed systematically following oral or topical administration. The effectiveness of any herbal product (or medication) is dependent upon delivering an effective level of the active compound.

The objective of the study is to establish the quantitative correlation between the photochemical, pharmacological profile and treatment of splenomegaly with a novel herbal dosage form which having better absorption, and produce better pharmacokinetic and pharmacodynamic profile than conventional herbal extracts.

By combining the emulsifying action of the phospholipids with standardized extract of *Tecomella undulata*, the Phytosome provides dramatically enhanced bioavailability for lipid soluble drugs explained by faster and improved absorption in the intestinal tract. Phytosome protect the valuable component of herbal extract from destruction by digestive secretion and gut bacteria.
1.6 Plan of work

1. Collection of Plant Material

2. Pharmacognostic Studies
   A. Macroscopically evaluation
   B. Microscopically evaluation
   C. Physiochemical studies
   D. Determination of extractive value

3. Preliminary Phytochemical Screening

4. Chromatography (HPTLC) Analysis

5. Pharmacological Screening

6. Preformulation Studies
   A. Spectroscopy methods
   B. Phytoconstituents– lipid interaction study

7. Formulation Development

8. Evaluation and Characterization
   A. Quantification of Tecomella undulata bark
   B. Size analysis of Phytosomes
   C. TEM
   D. Separation of entrapped and unentrapped plant extract
   E. Zeta Potential
   F. In-vitro Release studies

9. Stability Studies
   A. Short term
   B. Accelerated
Tree of Tecomella undulata

Flowering Branches of Tecomella undulata