PLAN OF WORK

The proposed plan of research work will be as follow:

Selection of herbal drugs on the basis of

- Literature studies
- Toxicity studies
- Dose

Collection and identification of herbal drug : Pharmacognostical evaluation of herbal drug

- Morphological identification of *A. Catechu*
- Microscopic studies of *A. Catechu*
- Chemical identification includes phytochemical screening tests for secondary metabolites (Flavonoids)
- Physical parameter such as total ash, water soluble ash, acid insoluble ash, sulphated ash, foreign organic matter, loss on drying, water and alcohol soluble extractive value.

Extraction and characterization of *A. Catechu* herbal extract

- Preparation of extract
- Determinations of Water and Alcohol soluble extractive value.

Isolation and characterization of Flavonoids from *A. Catechu*

- Isolation using column chromatography.
- Comparison with marker compound using TLC & HPTLC

Preparation of phytosomes

- Mechanical Dispersion Method
- Antisolvent precipitation technique
- Solvent evaporation technique

Characterization and evaluation of phytosomes

- Characterization e.g. visualization, vesicle size & zeta potential, entrapment efficiency, transition temperature, surface tension measurement, vesicle stability & drug content etc.
- Spectroscopic evaluation e.g. $^1$H-NMR, $^{13}$C-NMR, FTIR etc.

P. cokinetic study of optimized Phytosome

- Pharmacokinetic studies on optimized Phytosome will be carried out for assessment of bioavailability by using suitable animal model
Evaluation of pharmacodynamic activity: Pharmacodynamic evaluation of prepared Phytosomes for below mentioned activities by using suitable models

- Antibacterial activity
- Anti-oxidant activity
- Anti-fungal activity
- Anti-inflammatory activity
- Anti-secretory and Anti-ulcer Activity
- Anti-cancer activity

Compilation of data/Publications/ Patents etc.
RESEARCH METHODOLOGY:

The extracts of *A. catechu* exhibits various pharmacological effects like antipyretic, anti-inflammatory, anti-diarrhoeal, hypoglycemic, hepatoprotective, antioxidant and antimicrobial activities. From the heartwood of *A. Catechu* Freudenberg et al. have isolate (+)-catechin (dl) – catechin, and (-) epicatechin. Later, Rao and Seshadri reported (-)-epicatechin in a yield of 5% from the heart wood of *A. Catechu*. Perkin detected quercetin in the acacia catechu extract. Hatchway and Seakins reported the presence of quercetagetin and fisetin, chromatographically indistinguishable from each other. Further on Seshadri et al. reported the isolation method for catechin, gallocatechin, dicatechin and catechin tetramer as their methyl esters from the *A. Catechu* on the basis of very inadequate evidence. Heartwood of *A. Catechu* is rich in phenolics and flavonoids. Previously the acetone extractives of the heart wood were extracted with hexane, benzene, choloroform, ether and ethyl acetate. Extract was objected to repeated column chromatography (polyamide and silica gel) and subsequent PLC, this resulted in the isolation of kaemferol, dihydrokaepferol, isorhamnetin, ouercetin, taxifolin, (+)- Afzelchin, and (-)- epicatechin, and these compounds were characterised on the basis of UV, IR, NMR, Mass spectral data as well as by direct comparison with authentic sample by thier melting point, and superimposable IR spectra. All these compounds, except (-)-epicatechin, rest all have been isolated for the first time from *A.Catechu* heartwood.

**Extraction process of Active flavonoids in Acacia Catechu:**

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Acacia Catechu Heatwood is cut into chips and powdered

Powdered Drug Extracted with cold acetone

Actone extractive is extracted with number of solvent
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Brief outlines of A. Catechu Phytosome synthesis:

- Ether Extract
  - Contained wax and terpenoids
  - Not to be examined

- Seperation of product in ether extract carried out by column chromatography on polyamide
  - Compound (free flavonoids) were isolated with close Rf value
  - Dihtdrokaempferol, Isorhamnetin, Quercetin, Kaempferol, Taxifolin, Afzelchin, Epicatechin etc.

- Ethyl acetate extract
  - Epicatechin Procyanidin

Hexane, Benzene & Chloroform extract
Method used for preparation of phytosome:

- Mechanical Dispersion Method
- Antisolvent precipitation technique
- Solvent evaporation technique

**Mechanical Dispersion Method**

Phytosome Complex can be prepared by mechanical dispersion method. 100 mg weighed soy lecithin was dissolved in 2 ml of diethyl ether in beaker and put into bath sonicator. 50 mg of drug was dissolved in 20 ml double distilled water and this solution was added drop by drop into the beaker containing soy lecithin while sonicating and then left for 15 min for further sonication. The resultant formulation was kept in refrigerator.

**Antisolvent precipitation technique**
Specific amount of drug and phospholipid were refluxed with suitable solvent. The mixture so formed was concentrated and another solvent was then added for precipitation with continuous stirring. Precipitates thus formed were then filtered and collected and stored in vacuum desiccators overnight.

**Solvent evaporation technique**

The specific amount of phytoconstitents and soya lecithin were taken into a round bottom flask and refluxed with acetone at a temperature 50 – 60°C for 2 h. Precipitates thus formed were then filtered and collected and stored in vacuum desiccators overnight.

**Selection best formulation method**

Phytosomes were prepared by three different methods. Particle size, entrapment efficiency and % yield was calculated. After comparison of results of all the three methods, one is selected on the basis of Results of % Yield, Entrapment efficiency and Particle Size are summarized in following Table :

**Characterization of phytosome:**

- Visualization: Visualization of phytosomes can be achieved by scanning electron microscopy.
- Vesicle size: The particle size of phytosomes can be determined by dynamic light scattering which uses a computerized inspection system and photon correlation spectroscopy.
- Transition temperature: The transition temperature of vesicular lipid system can be determined by differential scanning calorimetry.
- Entrapment efficiency: The entrapment efficiency of a phytosomal formulation can be determined by subjecting the formulation to ultracentrifugation technique.
- Drug content: The drug contents will be measured with the help of UV-visible spectrophotometer.
- Zeta potential: it is measure by the use of zeta meter.

<table>
<thead>
<tr>
<th>Sr. No</th>
<th>Method</th>
<th>% Yield</th>
<th>Particle Size (nm)</th>
<th>Entrapment efficiency (%)</th>
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</thead>
<tbody>
<tr>
<td>1</td>
<td>Antisolvent precipitation technique</td>
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<tr>
<td>2</td>
<td>Solvent evaporation technique.</td>
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<tr>
<td>3</td>
<td>Mechanical dispersion method</td>
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</tbody>
</table>
- Vesicle stability: The stability of vesicles can be determined by assessing the size and structure of the vesicles over time. The mean size is measured by DLS and structural changes are monitored by TEM.

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Work</th>
<th>Periods</th>
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<tbody>
<tr>
<td>1.</td>
<td>Literature survey</td>
<td>5 month</td>
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<tr>
<td>2.</td>
<td>Selection of Herbal Drug</td>
<td>1 month</td>
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<td>3.</td>
<td>Pharmacognostical Evaluation of Acacia Catechu as a Herbal Drug</td>
<td>3 month</td>
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<td>4.</td>
<td>Extraction and Characterization of Acacia Catechu Herbal Extract</td>
<td>3 month</td>
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<td>5.</td>
<td>Isolation and Characterization of Flavonoids from Acacia Catechu</td>
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<td>6.</td>
<td>Preparation of Phytosome</td>
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<td>7.</td>
<td>Characterization and Evaluation of Phytosome</td>
<td>3 month</td>
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<td>8.</td>
<td>P.cokinetic study of optimized Phytosome</td>
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<td>9.</td>
<td>Evaluation of pharmacodynamic activity</td>
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<td></td>
<td>Compilation of data/Publications/ Patents etc.</td>
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<td></td>
<td><strong>Total duration of project</strong></td>
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