MATERIALS AND INSTRUMENTS USED

4.1. Materials

4.1.1. Drug

1. Theophylline [HIMEDIA Laboratories. Pvt. Ltd.,]
2. Salbutamol sulphate [Fourrts (India) lab Pvt Ltd, Chennai]

4.1.2. Polymers

1. Hydroxy propyl cellulose [Fourrts (India) lab Pvt Ltd, Chennai]
2. Sodium alginate [SD Fine chemicals, Boisar]

4.1.3. Other materials

1. Acetone [Nice chemicals Pvt Ltd, Cochin]
2. Dicalcium phosphate [Indian research products, Chennai]
3. Magnesium stearate [Molychem, Chennai]
4. Talc [Molychem, Chennai]
5. Barium sulphate [Indian research products, Chennai]
6. Seeds of *Caesalpinia pulcherrima* and *Leucaena leucocephala* are collected from the surroundings of nandyal, Andhra pradesh.
7. Distilled water.
4.2. Plant profile

4.2.1. Caesalpinia pulcherrima

Binomial name  
*Caesalpinia pulcherrima* (L.) Sw.

Synonym  Poinciana pulcherrima

Kingdom  Plantae

Subkingdom  Tracheobionta

Division  Mangnoliophyta

Class  Magnoliopsida

Subclass  Rosidae

Order  Fabales

Family  Fabaceae (alt. Leguminosae)

Subfamily  Caesalpinioideae

Tribe  Caesalpinieae

Genus  *Caesalpinia*

Species  *Pulcherrima*

Common names  Dwarf poinciana, Pride-of-barbados, Barbados flower-fence.

Habit  shrub

Description  "An erect, smooth shrub or small tree, 1.5-5 meters height. The branches are armed with a few scattered spines. Leaves are bipinnate, 4-8 pairs, 6-12 cm long. Leaflets are stalkless, 7-11 pairs, elliptic, and 1 or 2 cm long. Flowers are
red and yellow, borne on terminal racemes, about 4 cm in diameter. Petals are crisp and clawed. The pod is nearly straight, flat, smooth, 5-8 cm long and 1.5 cm wide, containing 6-8 seeds”.

![Fig. 4.1: Caesalpinia pulcherrima plant](image1)

![Fig. 4.2: Seed of Caesalpinia pulcherrima](image2)

**Distribution**  
California, Texas, Arizona, Florida and India.

**Chemical constituents**  
Leaves contain gallic acid, gum, tannin, resin and salts.

**Parts utilized**  
Roots, leaves, flowers, and bark.

**Folkloric uses**

a. Decoction of roots and leaves is used to cure fever.

b. Infusion of the bark used as wash for the teeth and gums.

c. Infusion of leaves used for colds, fevers, skin ailments and purging.

d. Decoction of leaves used as mouth wash and gargle for mouth ulcers.

e. Decoction of flowers used for erysipelas and inflammation of the eyes.

f. Fruit is astringent and used for diarrhea and dysentery.

g. The seeds cure bad cough, breathing difficulty, and chest pain.

h. 4 gms from the root is also said to induce abortion in the first trimester of pregnancy.
Summary at a glance

Table 4.1: Description about *Caesalpinia pulcherrima* plant

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Whole seeds*</th>
<th>Seed nuts*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Size (height x width)</td>
<td>6 feet x 6 feet</td>
<td></td>
</tr>
<tr>
<td>Flower color</td>
<td>Orange-red</td>
<td></td>
</tr>
<tr>
<td>Flower season</td>
<td>Summer - fall</td>
<td></td>
</tr>
<tr>
<td>Exposure</td>
<td>Full sun</td>
<td></td>
</tr>
<tr>
<td>Water</td>
<td>Moderate</td>
<td></td>
</tr>
<tr>
<td>Growth rate</td>
<td>Fast</td>
<td></td>
</tr>
<tr>
<td>Hardiness</td>
<td>Root hardy to 15º F.</td>
<td></td>
</tr>
<tr>
<td>Hardy in these cities</td>
<td>Tucson, Phoenix, San diego.</td>
<td></td>
</tr>
</tbody>
</table>

Table 4.2: Composition of whole seeds and seed nuts of *Caesalpinia pulcherrima*

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Whole seeds*</th>
<th>Seed nuts*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moisture %</td>
<td>9.05 ± 0.05</td>
<td>7.30 ± 0.1</td>
</tr>
<tr>
<td>Dry matter %</td>
<td>90.95 ± 0.05</td>
<td>92.7 ± 0.1</td>
</tr>
<tr>
<td>Ash content %</td>
<td>4.50 ± 0.11</td>
<td>6.22 ± 0.1</td>
</tr>
<tr>
<td>Organic matter %</td>
<td>95.50 ± 0.11</td>
<td>93.78 ± 0.1</td>
</tr>
<tr>
<td>Carbohydrates %</td>
<td>39.10 ± 0.1</td>
<td>18.3 ± 0.7</td>
</tr>
<tr>
<td>Crude protein %</td>
<td>48.08 ± 0.48</td>
<td>42.97 ± 0.37</td>
</tr>
<tr>
<td>Calorie value (kcal/100g)</td>
<td>312.15</td>
<td>217.47</td>
</tr>
</tbody>
</table>

* Mean ± Standard Deviation (n=3).

Table 4.3: Mineral composition in *Caesalpinia pulcherrima* whole seeds

<table>
<thead>
<tr>
<th>Mineral (mg/100g)</th>
<th>Whole seeds*</th>
<th>Seed nuts*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium (Na)</td>
<td>49.50 ± 0.05</td>
<td>40.50 ± 0.05</td>
</tr>
<tr>
<td>Potassium (K)</td>
<td>39.50 ± 0.05</td>
<td>31.00 ± 0.01</td>
</tr>
<tr>
<td>Calcium (Ca)</td>
<td>37.50 ± 0.03</td>
<td>30.50 ± 0.15</td>
</tr>
<tr>
<td>Magnesium (Mg)</td>
<td>58.50 ± 0.35</td>
<td>69.50 ± 0.05</td>
</tr>
<tr>
<td>Iron (Fe)</td>
<td>21.0 ± 0.01</td>
<td>15.0 ± 0.05</td>
</tr>
<tr>
<td>Phosphorous (P)</td>
<td>56.0 ± 0.04</td>
<td>124.0 ± 0.01</td>
</tr>
<tr>
<td>Na/K</td>
<td>1.23</td>
<td>1.30</td>
</tr>
<tr>
<td>Ca/P</td>
<td>0.66</td>
<td>0.25</td>
</tr>
</tbody>
</table>

Mean ± Standard Deviation (n=3).  
Na/K = Sodium to Potassium ratio ; Ca/P = Calcium to Phosphorus ratio.
4.2.2. Leucaena leucocephala.

**Binomial name**  
*Leucaena leucocephala* (Lam.) de Wit

**Synonym**  
Mimosa leucocephala Lamark

**Kingdom**  
Plantae

**Subkingdom**  
Tracheobionta

**Division**  
Magnoliophyta

**Class**  
Magnoliopsida

**Subclass**  
Rosidae

**Order**  
Fabales

**Family**  
Fabaceae

**Subfamily**  
Mimosoideae

**Tribe**  
Mimoseae

**Genus**  
Leucaena

**Species**  
*L. leucocephala*

**Common names**  
Chinese Petai

**Habit**  
Shrub

**Description**

Small plant up to 8 m high; leaves alternate, twice compound, 15-25 cm, base of petiole enlarged, leaflets 9-18 pairs, 7-12 mm long, linear-oblong, unequilateral, flowering stalks auxiliary, 3.5 to 5 cm long; flowers in dense globule heads 2 to 3 cm
in diameter, white, fruit a pod, strap-shaped, flattened, 12-18 cm long, 1-2 cm wide, papery, green turning brown and splits open along two edges when mature, several fruits develop from each flower head, seeds obviate, 5-8 mm long, 3-5 mm wide, shiny, brown. 

Chemical constituents

Fat-8.68%, crude fibre- 22.59%, nitrogen-free material other than fibre-9.78%, nitrogen-6.42%, sucrose, water-14.8%, ash-4.2%, tannins, carbohydrates.
Parts utilized  Dried seeds

Product uses

As food

Pods, seeds and leaf tips have been used as food, although mimosine toxicity makes this practice risky. In Indonesia, a food called ‘tempe lamtoro’ is made of fermented *Leucaena* seeds. Seeds can also be prepared as a coffee substitute.

As gum

Gum arises from *Leucaena* stems under ill-defined conditions of injury and disease or from sterile hybrids, especially *Leucaena leucocephala*, *Leucaena esculenta*.

Folkloric uses

Intestinal parasitism

Used against ascaris and trichinosis.

Dose

Adults

1 teaspoon of powdered dried seeds, alone or mixed with condensed milk and followed by half a glass of water, taken as a single dose 2 hours after a meal, repeated after one week as needed.

Children

7-8 years old-1/4 to 1/2 teaspoon, 9-12 years old-1/2 to 2/3 teaspoon.

Adverse effects

Abdominal pain, diarrhoea.
4.3. Polymer profile

4.3.1. Hydroxy propyl cellulose (HPC)

**Description**: It is a white to slightly yellow colored, odorless and tasteless powder.

**Synonyms**: HPC, pm50, LHPC, KLUCEL, Synthetic, Hyprolose, pm50(polynomial), hydroxypropyl, (2% in Water at 20°C), VE (Vitamin E Acetate Oil).

**Chemical name**: Hydroxy propyl cellulose

![Fig. 4.6: Structure of HPC](image)

**IUPAC Name**: Cellulose 2-hydroxypropyl ether oxypropylated cellulose.\(^{18} - ^{19}\)

**Molecular weight**: 59.087

**Properties**

1. Appearance: white or white-like fibre or granule powder.

2. pH value: 5.0～8.5.

3. Soluble in many organic solvents.

4. Aqueous solution of HPC shows smooth fluidity.

**Applications in pharmaceutical formulations**

1. Hydroxypropyl cellulose is widely used in oral and topical pharmaceutical formulations.

2. HPC is primarily used in tableting as a binder, film coating and extended release matrix former.
3. Concentrations of 15-35% w/w HPC may be used to produce extended release tablets.

4. The addition of anionic surfactant similarly increases the viscosity of HPC there by decreases the release rate of the drug.

5. HPC is also used in micro encapsulation process as a thickening agent.

**Stability and storage**: HPC powder is stable material. Aqueous HPC solutions have optimum stability at pH 6.0-8.0. Ultraviolet light will decrease the stability of HPC. Hydroxy propyl cellulose powder should be stored in a well closed container in a cool and dry place.

**4.3.2. Sodium alginate**

**Description**: The chemical compound sodium alginate is the sodium salt of alginic acid. Sodium alginate is a gum, extracted from the cell walls of brown algae. It occurs as white to yellowish brown filamentous, grainy, granular or powdered forms.

**Synonyms**: Sodium alginate, Landalgine, Nouralgine, Pectalgine, Algilne, Calginate, Combinace, Duckalgin, Kaltostat, Kelcosol, Protnal, Proratek, Amnucol, Halltex, Kelacid, Kelsize, Kelkone, Lamitex, Manucol, Manutex. 20-22

**Empirical Formula**: NaC₆H₇O₆.

![Structure of Sodium alginate](image)

**Fig. 4.7: Structure of Sodium alginate**

**IUPAC Name**: 6-(2-carboxy-4,5-dihydroxy-6-methoxyoxan-3-yl)oxy-4,5-dihydroxy-3-methoxyoxane-2-carboxylic acid
**Molecular weight** : 398.32

**Solubility** : Freely soluble in water, insoluble in ethanol and ether.

**Loss on drying** : Not more than 15% (105°C, 4h)

**Water insolubility** : Not more than 2% on the dried basis.

**Density** : 1.601 g/cm³

**Acidity (pKa)** : 1.5-3.5

**Properties**

1. Good surface properties.
2. Shows good elastic properties
3. Not dimensionally stable on storing due to evaporation.
4. Non toxic and non irritant.

**Uses**

1. **As moulage materials for dentistry**: In the past, moulage for dentistry is made of rubber and gesso which are substituted by Sodium alginate moulage materials with simple operation and accurate tooth form.

2. **As haemostatic**: Fiber type sedimentation of sodium alginate appears in acid or in calcium salt solutions. The molecular structure is in the form of thread. Therefore it is used in different haemostatics such, as hemostatic gauze, hemostatic sponge, scalding gauze and spraying haemostatic.
3. **In toxicity treatment**: Sodium alginate is used in toxicity of radio strontium. It prevents the absorption of the strontium in the GIT and hence it is discharged unabsorbed.

4. **In pharmaceutical formulations**: Sodium alginate is water soluble. The aqueous solution is viscous and jelly. It is used as thickening and suspending agents in pharmaceutical formulations.

**4.4. Drug profile**

**4.4.1. Theophylline**

![Structure of Theophylline](image)

**Fig. 4.8: Structure of Theophylline**

- **Molecular formula**: $\text{C}_7\text{H}_8\text{N}_4\text{O}_2$
- **Molecular weight**: 180.17 (anhydrous)
- **IUPAC Name**: 1,3 dimethyl 2,6 (1H, 3H) purinedione

Theophylline is prescribed to treat breathing problems (wheezing and shortness of breath) caused by asthma, bronchitis, or emphysema. It relaxes the smooth muscle of the bronchial airways (breathing tubes), which opens the air passages to the lungs and thereby allows air to move in and out more easily.\cite{23,34}

**Description**

Theophylline is a white, crystalline or amorphous powder. It’s an odourless, bitter compound.
Solubility

It is slightly soluble in water and sparingly soluble in ethanol and also soluble in alkali hydroxides, ammonia and mineral acids.

Action and use

Used as a bronchodilator.

Melting range: Between 269° C and 274° C

Light absorption

Extinction of a 1-cm layer of a 0.001% w/v of solution in 0.1 N HCl at 271 nm not less than 0.53, calculated with reference to the dried substance.

Pharmacokinetic data

Bioavailability : 100%
Protein binding : 40% primarily to albumin
Metabolism : Hepatic to 1- methyl uric acid
Half-life : 6 - 8 hrs

Dose

4 mg/kg/day orally.

Storage

Store theophylline in a tightly closed, light-resistant container at room temperature.

Side Effects

Minor

Diarrhoea, dizziness, flushing, headache, heartburn, increased urination, insomnia, irritability, loss of appetite, nausea, nervousness, or vomiting. These side effects shall disappear over time as the body adjusts to theophylline.
Major

Black, tarry stools, confusion, convulsions, difficulty in breathing, fainting, muscle twitches, palpitations, rash, severe abdominal pain, or unusual weakness.

Mode of action

1. Relaxes bronchial smooth muscle.
2. Increases rate and force of cardiac contraction.
3. Increases rate of urine production.

Pharmacokinetics

i. Administration and Absorption

Administered as slow i.v. injection/infusion or as tablets. Absorption is usually rapid and complete. Theophylline is relatively insoluble in biological fluids. To enhance the solubility of theophylline it is converted in to Aminophylline by the addition of ethylenediamine.

ii. Elimination

Ninety percentage of Theophylline is metabolized by liver. There is evidence that the process is saturable at therapeutic doses. Biological half life \( t_{1/2} \) is about 8 h. The half life is prolonged in patients with severe cardiovascular disease and in cirrhosis.

Clinical Uses: Treatment for chronic and acute asthma.

Adverse Effects

i. Nausea and diarrhoea at high therapeutic levels.

ii. Cardiac arrhythmias and fits when plasma concentration exceeds recommended range.
**Drug Interactions**

i. Reduced rates of theophylline elimination due to enzyme inhibition by erythromycin, ciprofloxacin, allopurinol, oral contraceptives

ii. Enhanced elimination due to enzyme induction by carbamazepine, phenobarbitone, phenytoin.

**4.4.2. Salbutamol sulphate**

![Structure of Salbutamol sulphate](image)

**IUPAC name:** 1-(4-hydroxy-3-hydroxymethylphenyl)-2-(t-butilamino)ethanol Sulphate.

**Molecular formula:** \((C_{13}H_{21}NO_3)_2H_2SO_4\)

**Molecular weight:** 288.35

**Description:** White or white crystalline powder, odourless.

**Solubility:** Freely soluble in water, slightly soluble in ethanol (95%) and in ether, very slightly soluble in dichloromethane.

**Category:** Beta adreno receptor agonist, tocolytic.

**Biological half-life:** 2-7 h.

**Dose:** Oral dose of salbutamol sulphate is equivalent of 4-16 mg of salbutamol, daily in divided doses.\(^{35,36}\)

**Storage:** It is stored in a well closed, light resistant container.

**pH range:** 3.4 - 5.0
Analytical parameters

Identification: Salbutamol sulphate at a concentration of 0.008% w/v in 0.1 M hydrochloric acid shows a maximum absorption at 276 nm.

Assay: Weigh accurately about 0.4gm of Salbutamol sulphate, dissolve in 5 mL of anhydrous formic acid, add 35 mL of anhydrous glacial acetic acid. The mixture is titrated against 0.1 M perchloric acid by potentiometrically. Each mL of 0.1 M perchloric acid is equivalent to 0.05767 g of salbutamol sulphate.

Pharmacology

i. Effect on electrolytes and metabolism: The most prominent effects that may be encountered are hypokalaemia. Degree of effect will often be depends upon the dose and route of administration.

ii. Effects on eyes: Salbutamol to a greater extent has been implicated in retinopathy in the premature infants when used for premature labour.

iii. Effect on heart: Adverse cardiac effect like tachycardia, some time occurred in persons using inhalers.

iv. Effect on mental functions: Visual hallucinations lasting for an hour have been reported following administration of nebulized salbutamol to elderly patient.

v. Effect on respiratory system: Paradoxical bronchoconstriction has been reported following bronchodilating therapy with nebulized solution of salbutamol apratropium bromide over dosage. Reports of salbutamol over dosage are tachycardia, Central nervous system (CNS) stimulation, tremors, hypokalemia and hyperglycemia.

vi. Pregnancy and neonates: Most adverse effects associated with salbutamol in pregnancy relate to the cardiovascular and metabolic effects of very high doses given via i.v. infusion.
Interaction with other Drugs

Hypokalemia is known to be possible side effect during the treatment with salbutamol and this may be enhanced during diuretic therapy. Salbutamol given by $i.v$ has been reported to enhance neuromuscular blockade produced by pancuronium and vecuronium.

4.5. Instruments used for the present investigations

1. RP-HPLC - Shimadzu SCL-10 Avp.

2. UV-Visible Spectrophotometer - Schimadzu UV 1201.

3. Electronic balance - Shimadzu BL 2204.

4. Rotary tablet punching machine (9 stations - Chamunda Pharmacy machinery Pvt. Ltd.).

5. 8 Basket digital dissolution apparatus USP XXI Paddle (Electro Lab-Model EDT-08 LX).


7. DSC - Model DSC-50 Shimadzu Automatic Analyzer.

8. NMR – Bruker-II 600 Spectrometer.

4.5.1. Other instruments

1. Monsanto tablet hardness tester.

2. Screw gauge (thickness tester).

3. Roche friabilator.

4. Ostwald’s viscometer.

5. Melting point apparatus - Lab India (MR-vis).

6. Hot air oven - INLAB Equipments, Madras.

7. pH meter - Elico LI-120.