CHAPTER 3

LITERATURE REVIEW
Section A: Nimbatiktam

3.1. Ingredient of Nimbatiktam  
Alcoholic Neem oil extract

3.2. Method of preparation as per SOP's supplied by CCRAS vide File number F.9-4/2005/PRI/TVM/Tech Dated 17th September 2008 (Letter attached as Annexure-II) (Mfg Date: November 2007; Shelf Life: 5 years)

Nimbatiktam is isolated from the neem oil of *Azadirachta indica*. Neem oil is extracted from neem seed by pressure.

Neem oil and rectified spirit are mixed in the ratio 1:1 by volume in hard glass bottle and horizontally shaken for 10-15 minutes in a mechanical shaker. The mixture is poured into a separating funnel. The alcohol layer is separated and collected. The oil layer is again shaken with rectified spirit as before and the process repeated for two or three times. The combined alcohol extract is concentrated by distillation to small volume. The extract us transferred into a separating funnel and shaken with petroleum ether to remove traces of oil if any. The alcohol layer is collected in a distilling flask and concentrated in a vacuum flash evaporator. The semi solid obtained is poured into cold water when it gets solidified. The solid is cut into small pieces and washed several times with cold water with stirring. The solid is ground well into powder, which is separated by filtration, washed with water and air dried.

3.3. Taxonomy of neem

Order  Rutaales  
Suborder  Rutinae  
Family  Meliaceae  
Subfamily  Melioidae  
Tribe  Meliaceae  
Genus  *Azadirachta*  
Species  *indica*

3.4. Description of neem oil

Neem oil is obtained by expression of seed of *Azadirachta indica* belongs to family Meliaceae (Kumar *et al.*, 2002). It is light to dark brown in colour with strong odour (combine with odours of peanut and garlic) and bitter in taste (due to presence of triglycerides and large amount of triterpenoids). Neem oil, bark of neem and leaf extracts of neem have been therapeutically used as folk medicine to control leprosy, intestinal, helmenthiasis, respiratory disorders, constipation and also as general health promoter. A number of chemical constituents and secondary plant metabolites, such as fatty acids, sterols, flavonoids, phenolics and carbohydrates have been isolated and
were characterized from neem oil (Kirtikar and Basu, 1975). Crude neem oil has also been reported to have antipyretic activity (Murthy and Sirsi, 1958), antidiabetic, antimicrobial, antifungal, antiviral (Sankaram et al., 1986) and immunomodulatory (Upadhyay et al., 1992). Several limonoids have also been identified, recently. Among them nimbolide, epoxazadirachtin, salannin, nimbin, deacetylnimbin, azadirachtin are of main concern (Cohen et al., 1996). Neem oil and its formulations are the most important of the commercially available neem products in the market.

3.5. Biological activity of neem oil

Many compounds (protomeiliacin, meiliacin, pentanortriterpenoid, hexanortriterpenoid, and nortriterpenoidal components like fatty acids, sterols, phenols, flavonoids and carbohydrates) have been isolated and characterized from neem seed oil (Kirtikar and Basu, 1975). Various activity of neem oil can be summarized as follows:

3.5.1. Insect repellent activity

Neem oil has repellent action against sand fly Phlebotomus argentipes and other mosquito species (Sharma and Dhiman, 1993). Study also carried out by burning kerosene oil mixed with neem oil (1%) against anopheles and culex species (Sharma and Ansari, 1994). It also has repellent activity against other malarial vector like Anopheles subpictus, Anopheles culicifacies, (Dua et al., 1995, Ansari and Razdan, 1996) and Anopheles darlingi (Moore et al., 2002). Neem cream also found to have repellent activity. Coconut oil mixed in 2% neem oil provided 96-100% protection to anophelines, 85% to Aedes, 37.5% to Armigeres and 61-94% against Culex spp (Sharma et al., 1995). Neem oil mixed with coconut oil when applied to exposed part of human volunteer had 81-91% protection during 12 h period (Mishra et al., 1995). It also showed repellent activity against female sandfly, Phlebotomus papaiasi (by applying 1% and 2% concentrations of neem oil) which is higher than standard N, N-diethylphenyl acetamide (DEPA) (Srinivasan and Kalyanasundaram, 2001). Neem oil at dose of 5 mL/person/night gave 50 and 40.9 % protection in indoor collections and 17.4 and 5.6 % in outdoor collection against Culex quinquefasciatus (Ravindran and Kar, 2002). It showed repellent and antifeedant activity against Culicoideles nubeculosus (Meigen) female by using Y-tube olfactometer (Blackwell et al., 2004), anti-tick repellent activity against nymphs of Ixodes ricinus, (Garboui et al., 2006).

3.5.2. Insecticidal/Molluscicidal property
Time and dose dependent molluscicidal property of leaf bark, cake, neem oil and the neem based pesticide, achook and nimbecidine was reported (Singh et al., 1996). The non-limonoids constituents of neem (6-hydroxy gedunin) with other limonoid constituents, gedunin, salannin, nimbine, and azadirachtin was found to have inhibitory activity against gram pod borer, Helicoverpa armigera (Hubner), and Asian armyworm, Spodoptera litura (Fabricius) (Koul et al., 2003). Neem oil shows 5% mortality in Japanese bettle (Popillia japonica) (Gupta et al., 2007) and louse infestation of angora goats Damalinia limbata (Phthiraptera) (Habluetzel et al., 2007).

2.5.3. Cytotoxic activity

Semisolid fractions of the neem seed oil have been found to have moderate to strong cytotoxicity with a possible involvement in mitochondrial pathway resulting in apoptotic death. The work is focused on the characterization of the effects of the neem oil components on cultured mammalian cells i.e. a mouse stabilized fibroblast line (3T6) used as a model system. It was observed a cytotoxic effect induced by the application of the oil extracts (Di Ilio et al., 2006).

3.5.4. Biodiesel

Biodiesel fluid (BDF), chemically monoalkyl fatty acid ester from non edible neem oil has been made by esterification (Nabi et al., 2006). It can be recycled and be used as renewable source of energy.

3.5.5. Anti-parasitic activity

Card board trap containing 20% neem oil causes 92% reduction of poultry red mite Dermanyssus gallinae (Lundh et al., 2005).

3.5.6 Reduction in environmental stress

Neem oil at 10 mg/L of causes ammonia removal from brackish water salinity (Krishnani et al., 2002). Neem oil also causes the mitigation of methane and nitrous acid emission from fertilized soil in rice wheat system in gangetic plains. Hence causes in reduction in global warming (Malla et al., 2005).

3.5.7. Antimicrobial activity

Bactericidal activities of neem seed oil have been studied against 14 strains of pathogenic bacteria by using dilution technique. They caused reduction in 21.42% at 500μL/mL, 7.14% at 250 μL/mL and 71.45 at 125 μL/mL. Activity was due to
inhibition of cell membrane proliferation. However, NIM-76 (spermicidal portion of neem oil) was found to be a broad spectrum bactericidal (Escherichia coli and Klebsiella pneumoniae), antifungal (Candida albicans), antiviral (Polio virus) and also in systemic candidiasis (Sairam et al., 2000).

A number of larvicidal (Table 3.1) spermicidal activities (Table 3.2) was also reported after application of various products of neem oil.
<table>
<thead>
<tr>
<th>Sr No</th>
<th>Compound/Formulation</th>
<th>Larvae of</th>
<th>Stage</th>
<th>Mechanism reported</th>
<th>References</th>
</tr>
</thead>
</table>
| 1.    | Neem oil (5% in acetone) | *Anopheles stephensi*  
*Aedes aegypti*       | Larval stage                | ----                      | Nagpal *et al.*, 1995     |
| 2.    | Neem oil emulsion (5% neem oil) | *Anopheles stephensi*  
*Culex quinquefasciatus*  
*Aedes aegypti*       | 3rd & 4th instar  
Pupal  
Population          | Toxicity                   | Batra *et al.*, 1998      |
| 3.    | Neem oil (100% concentration) | *Amblyomma variegatum*       | Larval Stage                | Toxicity to larvae        | Ndumu *et al.*, 1999       |
| 4.    | Neem oil                | *Paenibacillus larvae*       | ----                      | Physical and toxicological | Melathopoulos *et al.*, 2000a |
| 5.    | Neem oil                | *Acarapis woodi* (Rennie)  
(Honey bee parasite)          | Larval stage                | ----                      | Melathopoulos *et al.*, 2000b |
| 6.    | Neem oil                | *Varroa jacobsoni* (Oudemans)  
(Honey bee parasite)          | Larval stage                | ----                      | Melathopoulos *et al.*, 2000a |
| 8.    | Neem oil                | *Hyalomma anatolicum*  
excavatum                     | Hatching stage              | Effect on hatching rate of eggs | Abdel Shafy and Zayed, 2002 |
<p>| 9.    | Neem oil (5% emulsified) | <em>Cosmopolites sordidus</em>       | Egg, immature              | Larva take much longer time to live | Musabyimana <em>et al.</em>       |</p>
<table>
<thead>
<tr>
<th></th>
<th>(Germar)</th>
<th>and adult</th>
<th>locate feed</th>
<th></th>
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<tbody>
<tr>
<td>11.</td>
<td>Neem oil</td>
<td><em>Hyalomma dromedarii</em> (camel tick)</td>
<td>Larva and adult stage</td>
<td>Effect on egg production and feeding</td>
</tr>
<tr>
<td>12.</td>
<td>Neem oil</td>
<td><em>Frankliniella occidentalis</em> (western flower thrips)</td>
<td>Larvae</td>
<td>Thoeming et al., 2003</td>
</tr>
<tr>
<td>13.</td>
<td>Neemix</td>
<td><em>Culex quinquefasciatus</em></td>
<td>Last instar</td>
<td>Destruction of epithelial cell, Lysis of microvilli</td>
</tr>
<tr>
<td>14.</td>
<td>Neem powder</td>
<td><em>Culex quinquefasciatus</em></td>
<td>Last instar</td>
<td>Lysis of cell</td>
</tr>
<tr>
<td>15.</td>
<td>Formulated neem oil</td>
<td><em>Culex quinquefasciatus</em></td>
<td>Last instar</td>
<td>Disappearances of food column and epithelial cell destruction</td>
</tr>
<tr>
<td>16.</td>
<td>Neem oil Formulation</td>
<td><em>Anopheles gambia</em></td>
<td>3rd and 4th instar</td>
<td>----</td>
</tr>
<tr>
<td>17.</td>
<td>Nimbecidine/Neem gold</td>
<td><em>Lepidoccephalichthys guntea</em> (Hamilton Buchanan)</td>
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</tr>
</tbody>
</table>
### Table 3.2 Spermicidal Activity of Neem Oil

<table>
<thead>
<tr>
<th>Sr. No</th>
<th>Formulation and Route of administration</th>
<th>Test animals</th>
<th>Activity</th>
<th>Mechanism reported</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.</td>
<td>NIM-76 (Volatile fraction of the neem oil) <em>(in vitro)</em></td>
<td>Rat and Human spermatozoa</td>
<td>Antimotility effect</td>
<td>Inhibition of spermatozoal motility.</td>
<td>Riar <em>et al.</em>, 1990</td>
</tr>
<tr>
<td>5.</td>
<td>NIM-76 (Volatile fraction of the neem oil)</td>
<td>Rats</td>
<td>Antifertility</td>
<td>Spermicidal activity (Before coitus more effective than post coitus).</td>
<td>Riar <em>et al.</em>, 1991</td>
</tr>
<tr>
<td>7.</td>
<td>Neem oil <em>(in vitro)</em></td>
<td>Mice egg and sperm</td>
<td>Inhibition of <em>in vitro</em> fertilization (IVF)</td>
<td>Inhibition of 1st cleavage (2-celled embryo). Inhibition of blastocyst formation.</td>
<td>Juneja and Williams, 1993</td>
</tr>
<tr>
<td>No.</td>
<td>Treatment</td>
<td>Species</td>
<td>Effect</td>
<td>Description</td>
<td>Reference</td>
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<tr>
<td>8.</td>
<td>Neem oil (in the lumen of each vas deferens)</td>
<td>Male Rat</td>
<td>Pre coital antifertility</td>
<td>Blockage of spermatogenesis without affecting testosterone production.</td>
<td>Upadhyay et al., 1993</td>
</tr>
<tr>
<td>9.</td>
<td>Neem oil (Intrauterine administration)</td>
<td>Bonnet monkey</td>
<td>Reversible antifertility effect</td>
<td>Enhanced antigen presenting ability of the uterus.</td>
<td>Upadhyay et al., 1994</td>
</tr>
<tr>
<td>10.</td>
<td>Neem oil (in vitro)</td>
<td>Female mice</td>
<td>Post coital antifertility</td>
<td>Inhibition of 1st cleavage (2-celled embryo). Inhibition of attachment and proliferation of trophectodermal cells of partially hatching blastocysts.</td>
<td>Juneja et al., 1994</td>
</tr>
<tr>
<td>12.</td>
<td>Purified neem seed oil (Praneem vilci) (Intrauterine instillation/Phase 1 clinical trial)</td>
<td>Female volunteer</td>
<td>Antifertility</td>
<td>Leukocytes infiltration into the uterus.</td>
<td>Upadhyay et al., 1990</td>
</tr>
<tr>
<td>13.</td>
<td>Neem oil and its volatile fraction (Exposure/Contact)</td>
<td>Anopheles stephensi An. culicifacies</td>
<td>Impairment of gonotrophic cycle</td>
<td>Inhibition of oviposition due to absorption through the cuticle. Impaired vitellogenesis.</td>
<td>Dhar et al., 1996</td>
</tr>
<tr>
<td>14.</td>
<td>Neem oil (surgically injected into uterine horn post coitum)</td>
<td>Mice</td>
<td>Post coital fertility blocker</td>
<td>Lowering of epidermal growth factor receptor (EGFR) in lumen and glandular epithelium. Leukocytes infiltration into the uterus. Post implantation embryonic resorption</td>
<td>Juneja et al., 1996</td>
</tr>
</tbody>
</table>
## Chapter 3 Literature Review

<table>
<thead>
<tr>
<th></th>
<th>Literature</th>
<th>Effect on motility of sperm in a dose dependent manner and formation of pores and vesicles over the sperm head due to damage of cell membrane.</th>
<th>Sharma <em>et al.</em>, 1996</th>
</tr>
</thead>
<tbody>
<tr>
<td>15.</td>
<td>NIM-76 (Volatile fraction isolated from neem oil) (<em>in vitro</em>)</td>
<td>Human sperm</td>
<td>Spermicidal activity/Vaginal contraceptive</td>
</tr>
<tr>
<td>16.</td>
<td>Neem extracts (Orally at post implantation stage)</td>
<td>Rodents and primates</td>
<td>Termination of pregnancy at post implantation stage</td>
</tr>
<tr>
<td>17.</td>
<td>Neem extracts (Oral administration)</td>
<td>Rats/Baboons/Monkeys</td>
<td>Termination of pregnancy</td>
</tr>
</tbody>
</table>
3.5.8. Reported safety studies on neem oil

In early times, neem oil was found to have dose related toxicity symptom along with a number of histological and biochemical indices (Gandhi et al., 1988). However, researcher proved that only some damage in luminal epithelium of the uterus and decreased glycogen and total protein contents in the ovary and uterus can be correlated with neem oil (Tewari et al., 1989). Later on some scientist showed that debitterized neem oil is safe for consumption by human (Chinnasamy et al., 1993). Single application of 1% neem oil did not produce any skin irradiation (Valecha et al., 1996). It also showed non mutagenic activity without degradation of Azadirachtin A (Johnson et al., 2000). Ninety days daily oral crude neem oil treatment (5g/kg body weight) to the mice did not cause any significant changes in weekly body gain, serum/liver damage indicator, direct bilirubin or total bilirubin count (Awad, 2003). This was also devoid of toxicity, mortality or changes in tissue pathology (Raizada et al., 2001). The main constituent azadirachtin has not any adverse effect on respiratory system and enzymatic changes (AST, ALT) (Srivastava and Raizada, 2007).
3.6. Main chemical constituents of Nimbatiktam with possible mechanism against psoriasis

3.6.1. Nimbidin

Nimbidin was screened in comparison with two standard anti-inflammatory agents phenyl butazone a non steroids and prednisolone a steroid; against various experimental models of inflammation. Nimbidin significantly reduced the acute paw oedema in rats induced by phlogistic agents, carrageenin and kaolin and also significantly suppressed the formalin induced arthritis of the ankle joint and the fluid exudation in croton oil induced granuloma in rats. In acute phase of inflammation nimbidin (40 mg/kg), the study result revealed that nimbidin was effective in both acute and chronic phases of inflammation and can be considered as a general anti inflammatory agent (Pillai and Santhakumari, 1981).

Nimbatiktam is a mixture of tetranortriterpenes (nimbin, nimbidinin) and is prescribed 400 mg/day in divided dose. It has been studied clinically on diagnosed cases of psoriasis. The drug was administered in capsules form twice a daily for 60 days. There was significant improvement in symptoms like roughness; exfoliation etc. and about 87% of the patients got relieved from all these symptoms. More than 350 patients with well defined psoriatic lesions showed no complications during clinical trial. The 60% cases showed good control without any relapse. An Indian patent was granted 545/DEL/84 on Nimbatiktam for the management of psoriasis (Kitibha) (Nimbatiktam- 545/DEL/84; Kaur et al., 2004).

The bitter solid isolate, from the oil of seeds of Azadirachta indica (Meliaceae) found to possess potent anti-inflammatory and anti arthritic activity. The seed was found to contain approximately 45% oil which contains loeic acid (50-60%), palmitic acid (13-15%), stearic acid (14-19%), linoleic acid (8-16%), and arachidic acid (1-3%). Moreover, a number of other bitter components nimbin (0.12%), nimbinin (0.01%), and nimbidiol (0.5%) has also been identified. The crude neem oil was also reported to have antimicrobial, antifungal and antiviral effects (Mongkholkhajornslip et al., 2005). In another study, it was revealed that nimbidin extensively inhibited some of the function of macrophages and neutrophils related to the inflammatory response following both in vivo and in vitro experiment. Oral administration of 5-25 mg/kg of nimbidin to rats for three successive days significantly inhibited the migration of
macrophages to their peritoneal cavities in response to the inflammatory stimuli and also inhibited phagocytosis and phorbol-12-myristate13-acetate (PMA) stimulated respiratory burst in these cells. Moreover, nimbidin inhibited nitric oxide (NO) and prostaglandin E₂ (PGE₂) production in lipopolysaccharide (LPS) stimulated macrophages following *in vitro* exposure, whereas interleukin 1 (IL 1) was only weakly inhibited. The mechanism of NO inhibition revealed that nimbidin ameliorated the induction of inducible NO synthase (iNOS) without any inhibition in its catalytic activity. In addition, nimbidin also attenuated degranulation in neutrophils assessed in terms of release of β-glucoronidase, myeloperoxide and lysozyme. The result showed that nimbidin suppressed the functions of macrophages and and neutrophils relevant to inflammation. Therefore nimbidin can be helpful in treating inflammation/inflammatory disease especially in case of psoriasis and psoriatic arthritis (Kaur *et al.*, 2004)

### 3.6.2. Nimbin

Nimbin one of the bitter principle isolate of neem oil and closely related compound to nimbidin, nimbinic acid and nimbolide have been found to have anti-inflammatory, anti-pyretic and anti-arthritic properties and used to treat hypoglycaemia and peptic ulcer. (Tonthubthimthong *et al.*, 2001; Sidhu *et al.*, 2004; Tonthubthimthong *et al.*, 2004)

### 3.6.3. Azadirachtin and salannin

The role of azadirachtin, an active component of *Azadirachta indica* on TNF-induced cell signaling in human cell lines was investigated and found that it blocks TNF-induced activation of nuclear factor κB (NF-κB) with expression of NF-κB-dependent genes such as adhesion molecules and cyclooxygenase - 2. The *in silico* data suggest that azadirachtin strongly binds in the TNF binding site of TNFR and modulates cell surface TNFRs thereby decreasing TNF-induced biological responses. Thus, azadirachtin exerts an anti-inflammatory response by novel pathways (Thoh *et al.*, 2010; Thoh *et al.*, 2011; Akihisa *et al.*, 2009), which may be beneficial for anti-inflammatory therapy and can assist in managing the psoriasis A paste made of *Azadirachta indica* and *Curcuma longa* used to treat 814 people with scabies, a skin disease which cured 97% of them within three to five days of treatment. It was found that it has some active principles azadirachtin, salannin nimbin, and 6-desacytlnimbin (Lans, 2007)
Section B: Lajjalu Keram

3.1. Ingredients of Lajjalu Keram

<table>
<thead>
<tr>
<th>Description</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Coconut oil <em>(Coecus nucifera)</em></td>
<td>1 Part</td>
</tr>
<tr>
<td>2. Fresh Lajjalu plant juice <em>(Mimosa pudica)</em></td>
<td>4 Part</td>
</tr>
<tr>
<td>3. Kalka of leaf of fresh Lajjalu plant <em>(Mimosa pudica)</em></td>
<td>1/8 Part</td>
</tr>
</tbody>
</table>

3.2. Method of preparation Lajjalu Keram as per SOP’s supplied by CCRAS vide File number F.9-4/2005/PRI/TVM/Tech Dated 17th September 2008 (Letter attached as Annexure-II) (Mfg Date: November 2007; Shelf Life: 4 years)

Mixed the above items in a copper vessel and boiled. After boiling, stirred well continuously so that kalka is not allowed to adhere in the vessel and the oil is prepared in the low fire. When the kalka comes to kharapaka the oil is removed from fire and filtered. Then cooled and packed.

3.3. Taxonomy of *Mimosa pudica* (Source of Lajjalu Keram)

<table>
<thead>
<tr>
<th>Kingdom</th>
<th>Plantae</th>
</tr>
</thead>
<tbody>
<tr>
<td>Division</td>
<td>Magnoliophyta</td>
</tr>
<tr>
<td>Class</td>
<td>Magnoliopsida</td>
</tr>
<tr>
<td>Subclass</td>
<td>Rosidae</td>
</tr>
<tr>
<td>Order</td>
<td>Fabales</td>
</tr>
<tr>
<td>Family</td>
<td>Mimosaceae</td>
</tr>
<tr>
<td>Genus</td>
<td>Mimosa</td>
</tr>
<tr>
<td>Species</td>
<td>pudica</td>
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</tbody>
</table>

3.4. Common name of *Mimosa pudica*

<table>
<thead>
<tr>
<th>Language</th>
<th>Name</th>
</tr>
</thead>
<tbody>
<tr>
<td>English</td>
<td>Sensitive plant, sleeping grass</td>
</tr>
<tr>
<td>French</td>
<td>Sensitive</td>
</tr>
<tr>
<td>Latin</td>
<td><em>Mimosa pudica</em> Linn.</td>
</tr>
<tr>
<td>Hindi</td>
<td>Lajwanti, chui-mui</td>
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<tr>
<td>Bengali</td>
<td>Lajabati</td>
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<tr>
<td>Marathi</td>
<td>Lajalu</td>
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<td>Telugu</td>
<td>Attapati, peddanidrakanni</td>
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</tbody>
</table>
Chapter 3

3.5. Description of *Mimosa pudica*

*Mimosa pudica* Linn. is a widely distributed plant more or less in throughout of India (tropical and subtropical) (Chopra *et al.*, 1980). It is native to Brazil. It is short lived evergreen sub shrub and usually treated as an annual. The fern like leaves close up and droop when touched, usually re-opening within minutes. It has prickly stems and small, fluffy, ball shaped pink flowers in summer. The stem is erect, slender and branching. The leaves are bipinnate; fern like and pale green closing when disturbed. The flowers are pale lilac pink, occurring in globose heads and appearing in summer. Pods are crowded, flat, prickly and briskly. Seeds are bristles on seed pod cling to fur and clothing, about 2 mm broad, round and brown.

Indigenous to the northern hemisphere, it is adaptable to most soils in an open, sunny position, and is drought and frost tender. Due to its ability to fix nitrogen from the air it grows well on poor soils. "Sensitive Plant" folds up its leaves when touched or exposed to a flame. This plant requires a medium light exposure, an evenly moist soil, and temperatures between 60 and 85 degrees. One should use caution when handling seedlings because the plant dislikes root disturbance.

3.6. Chemical constituent of *Mimosa pudica*

The main bioactive constituent of this plant is an alkaloid Mimosine. The pipecolic acid, 5-hydroxy-pipecolic acid, ascorbic-acid, crocetin, crocetin-dimethyl-ether, D-glucuronic-acid, D-xylose, linoleic-acid, linolenic-acid, mucilage, norepinephrine, oleic-acid, palmitic-acid, sitosterol and stearic-acid has also been reported (Tiwari *et al.*, 1967). Studies showed the presence of cassiaoccidentalin B, two unusual C-glycosylflavones and 5-deoxyflavonoI derivative (Lobstein *et al.*, 2002; Kirk *et al.*, 2003).

**Mimosine**

Mimosine is a non-protein amino acid found in leaves, pods and seeds of tropical legumes of the genus mimosa. Mimosine is degraded in the rumen to 3-hydroxy-
4(1H)-pyridone (3,4-DHP), a goitrogen and hence consumption of plant in ruminants may cause poor growth, alopecia, swollen and raw coronets above the hooves, lameness, mouth and esophageal lesions, depressed serum thyrosine levels, and goiter. Susceptibility of ruminants to intoxication, however, greatly depends on specific microbial populations. Further, transfer of ruminant fluids from a resistant animal to a susceptible one results in a complete elimination of the toxic effects of Mimosine. This appears to be a possible solution to the toxicity in ruminant animals. (Maryendele, 2006).

**Synonyms**

α-Amino-3-hdroxy-4-oxo-1(4H) pyridine propanoic acid, 3-hdroxy-4-oxo-1(4H)-pyridinealanine, β-[N-(3-hydroxy-4-pyridone)]-α-amino paopionic acid, leucaenol, leucenol, leucenine, leucaenine, leucaenol

**Characteristics:**

A naturally occurring amino acid found in large quantity in the seed and foliage of the legume (genera Mimosa & Leucena).

**Molecular formula** C₈H₁₀N₂O₄

**M.P** 226-227°C

**Solubility**

- Slightly soluble in water
- Much less soluble in methanol & ethanol
- Practically insoluble in higher alcohol, dioxane, ethyl acetate, ether, benzene, chloroform, glacial acetic acid and pyridine
- Soluble in dilute acid or base
- **U.V. maxima** – 282 nm
- **dl-form** - Crystal from water.
- **dl-hemihydrate** - Crystal, darken at 215-226°C, M.P. (227-228)°C
- **l-form** - Crystal from water, M.P. (225°C), [α]D²²-20°C
- **Hydrochloride** - C₈H₁₁ClN₂O₄, Deo-175°C,
- **Hydrobromide** - C₈H₁₁BrN₂O₄, Deo-179°C
3.7. Analytical work reported on *Mimosa pudica*

A sensitive and selective spectrophotometric method for the estimation of the toxic factor Mimosine and 3-hydroxy-4-(1H) pyridone (DHP) has been reported based on the intense yellow colored azodye formed with p-nitroaniline, which showed a sharp absorption maximum at 400 nm. The method was optimized based on relative sensitivity of the reaction with various aromatic primary amino compounds and under different conditions of pH. Interference from a variety of structurally related compounds and phenols was tested and found insensitive to this method. The molar extinction coefficient at 400 nm for the azodye formed with Mimosine was $5.31 \times 10^4$ M$^{-1}$ cm$^{-1}$ and that for DHP was $1.699 \times 10^4$ M$^{-1}$ cm$^{-1}$. The applicability of the method was tested using different plant extracts and recovery was found to be at $100 \pm 0.3\%$. The method is suitable for accurate estimation of both mimosine and DHP after paper chromatographic separation of extracts from different biological samples (Lalitha *et al.*, 1993).

A simple, sensitive and quick spectrophotometric method for direct estimation of mimosine which involves measurement of absorbance of mimosine containing solution at 282 nm, the characteristic absorption maxima of mimosine. The method works satisfactorily under acidic and alkaline pH with pure mimosine standard as well as with biological samples in its crude form. Structurally related compounds and phenolic do not interfere with this method. Recovery analysis shown good results and can be used directly in crude biological sample (Verma *et al.*, 1993; Singh and Jabri, 2001).

A simple and quick spectrophotometric method for indirect estimation of Mimosine based on its reaction with diazotized sulphanilamide (DZSAM) was reported. DZSAM couple with N-(1-naphthyl) ethylenediamine (NEDA) forming pink colored azodye, absorbing maximally at 540nm ($e_{max} = 27$ mM $^{-1}$ cm$^{-1}$). The unreacted DZSAM was determined by DZSAM-NEDA-coupled with NEDA. The reaction of mimosine with DZSAM proceeded optimally at neutral pH. The decrease in absorption of the DZSAM-NEDA-coupled product obeyed Beer's law in the concentration range of 0.005-0.15 µg ml$^{-1}$ of mimosine. The present method was applied to estimate mimosine in plant extract containing lesser than 0.05 µg ml$^{-1}$ with recovery at 99±0.41% (Lalitha and Kulothungan, 2004).
A sensitive, simple and reliable reverse-phase HPTLC method has been reported for quantification of Mimosine in *Mimosa pudica* Linn. The plant powder was first extracted with methanol. The residue was then extracted with water and the aqueous extract was used for quantification. Chromatography was performed on silica gel RP-18 F<sub>254</sub> plates with ethyl acetate:glacial acetic acid:water, 6:1:1.7 (v/v) as mobile phase. Quantification was achieved by densitometric scanning at λ<sub>max</sub>=282 nm in reflectance-absorbance mode. The response to mimosine was a linear function of concentration over the range 30 to 100 µg mL<sup>-1</sup> in the extract. The amount of mimosine in *Mimosa pudica* was found to be 20 mg g<sup>-1</sup>. The method was validated for linearity, precision, accuracy, and robustness (Lakshmi *et al.*, 2007).

3.8. Pharmacological work reported on *Mimosa pudica*

The aqueous root extract of *Mimosa pudica* dose dependently inhibited the hyaluronidase and protease activities of Indian snakes (*Naja naja*, *Vipera russelli* and *Echis carinatus*) (Rosado-Vallado *et al.*, 2000).

The decoction of *Mimosa pudica* leaves at dose of 1000-4000 mg/kg protected mice against pentylentetrazol and strychnine-induced seizures in mice and had no effect against picrotoxin-induced seizures. It also antagonized N-methyl-D-aspartate-induced turning behaviour (Rastogi *et al.*, 2001).

Studies on the hormonal properties of the aqueous alcoholic and petroleum ether extracts of leaves of *Mimosa pudica* showed anti-estrogenic activity as the extracts inhibits significantly uterotrophic effect of estradiol-17β in mice. The anti-estrogenicity of the extract was corroborated by the depletion of glycogen content and alkaline phosphatase activity of the estrogen treated uterus. Extracts at 150 mg/kg orally upto 7 days of post coitum effectively inhibited implantation in rat (Valsala and Karpagaganapathy, 2002).

*Mimosa pudica* root extract was used to ascertain its efficacy as a male regulating agent in *Rattus norvegicus*. Two dose levels (100 and 150 mg/kg body weight) of the powered root extract were administrated orally to adult male albino rats to determine their effects on fertility. The cohabitation tests showed a reduced fertility rates in the high dose group (Girish *et al.*, 2004).

Aqueous and alcoholic extract of dried roots of *Mimosa pudica* were tested for their inhibitory on lethality, myotoxicity and toxic enzyme of *Naja kaouthia* venom. The
aqueous extract, particularly the normal water extract, displayed a significantly inhibitory effect on the lethality, myotoxicity and tested enzyme activities of venom compared with alcoholic extracts. The findings suggested that aqueous extracts of *Mudica pudica* root possess compound(s), which inhibit the activity of cobra venom (Bum *et al.*, 2004).

A clinical trial on patients with well-defined psoriatic lesion showed significant improvement in symptoms like roughness exfoliation and about 87% of the patient got relieved from all these symptoms after topical application of Lajjalu Keram (Anonymous, 2012).

The phytochemical studies has shown the presence of tannins, steroids, alkaloids, triterpenes, flavonoid glycoside, C-glycosylflavones, free amino acids, sitosterol, linoleic acid and oleic acid. Ayurveda describes *Mimosa pudica* for arresting bleeding and enhancing wound healing process (Bum *et al.*, 2004; Kokane *et al.*, 2009).

*Mimosa pudica* root powder (150 mg/kg, body weight) when administered intragastrically, altered the oestrous cycle pattern in female *Rattus norvegicus*. There was a significant reduction in the number of normal ova in rats treated with the root powder compared with the control rats, and a significant increase in the number of degenerated ova (Valsala, 2005).

Two patents have been granted for topical formulations containing Mimosine (WO/2007/129133 and EP20070704740) for the treatment of psoriasis either in the form of cream or ointment (Abel and Ruth, 2007, Moises *et al.*, 2008).
3.9. Main chemical constituents of Lajjalu Keram with possible mechanism against psoriasis

The synchronization effects of the plant amino acid mimosine on proliferating higher eukaryotic cells (HeLa or EJ30) cells showed that 0.5 mM Mimosine can induce a cell cycle arrest of human somatic cells in late G1 phase, before establishment of active DNA replication forks. The G1 phase arrest by 0.5 mM Mimosine was reversible upon mimosine withdrawal (Krude, 1999).

Exponentially growing mouse erythroleukemia MEL cells and quiescent human were treated with different concentrations of the nonprotein amino acid Mimosine for 16 h. The treatment of the cycling cell population with 400 mM Mimosine caused inhibition of DNA replication, changes in the progression of the cells in the cell cycle, and apoptosis. The rate of break accumulation was dose-dependent, did not depend on the stage of the cell cycle and was not connected with the mechanism of DNA replication. The data indicate that the effects of Mimosine on DNA synthesis and the cell cycle may be a result of introduction of breaks into DNA (Mikhailov et al., 2000).

It can be concluded that Lajjalu Keram acts locally by inhibiting early cell multiplication in psoriatic patients. Additionally, the coconut oil present in Lajjalu Keram smoothens the affected area and keeps the psoriatic wound moist.
Section C: 777 oil

3.1. Ingredients of 777 oil

1. Cocon oil \((Coccus~nucifera)\) 50% (v/v)

2. Leaves of \(Wrightia~tinctoria\) 50% (v/v)

3.2. Method of preparation of 777 oil as per SOP’s supplied by CCRAS vide File number F.9-4/2005/PRI/TVM/Tech Dated 17th September 2008 (Letter attached as Annexure-II) (Mfg Date: November 2007; Shelf Life: 4 years)

Leaves of \(Wrightia~tinctoria\) was taken in equal quantity of coconut oil. The fat soluble material of the leaves \(Wrightia~tinctoria\) are extracted into the coconut oil. Whole extraction was carried out in sunlight (Sooriya pudam). When all fat soluble part is extracted then extracted leaves are removed from oil and packed in bottle.

3.3. Taxonomy of \(Wrightia~tinctoria\) (Source of 777 oil)

Kingdom Plantae

Family \(Apocynaceae\)

Subfamily \(Apocynoideae\)

Tribe \(Wrightieae\)

Genus \(Wrightia\)

Species \(tinctoria\)

3.4. Common name of \(Wrightia~tinctoria\)

- Sanskrit: Hyamaraka
- Hindi: Indrajau, mithaindrajau
- Bengali: Indrajau
- Gujarati: Indrajau, runchallodudhlo, dudhlo
- Marathi: Kala kuda, indrajau
- Telugu: Tedlapaala, ankuda, jeddapaala
- Tamil: Veypale, irumpalai, thonthapalai
- Kannada: Kodamurki, beppale kodesige
- Malayalam: Kotakappalla, aiyapala
- Oriya: Pita~karuan~dudhokriya, krya
3.5. Description of *Wrightia tinctoria*

*Wrightia tinctoria* R. Br., Apocynaceae, is a small deciduous tree, generally up to 1.8 m tall and often 60 cm girth, sometimes up to 7.5 m high, distributed all over India. (Shruthi *et al.*, 2010). Bark of plant is light grey scaly smooth; leaves elliptic ovate oblong 7.5-12.5 cm long; flowers white, terminal cymes; follicles in pairs, pendulous, 15-50 cm long, cylindrical tips adhering; seeds linear, with basal coma.

The wood is uniformly white when first exposed, turning ivory-coloured with age; heartwood not distinct. The bark is commonly used as an adulterant of the well-known drug conessi, tellicherry or kurchi bark which is obtained from *Holarrhena antidysenterica*. The bark of *W. tinctoria* is very poor in alkaloidal content, the antidysenteric principle, and is easily distinguishable (Chopra *et al.*, 1958).

The bark and seeds are used in flatulence and bilious affections. A decoction of the leaves and bark is taken as a stomachic. The dried and ground bark is rubbed over the body in dropsy. The seeds are said to possess aphrodisiac and anthelmintic properties. The fresh leaves are very pungent and are chewed for relief from toothache. Alcoholic and aqueous extracts of the leaves and roots, as demonstrated on cats, possess hypotensive properties (Pritam and Bari, 2010).

The juice of the fresh unripe fruits is also used for coagulating milk, due to presence of a proteolytic enzyme. The seeds yield a deep red, semi-drying oil with the following fatty acid composition: linoleic, 31.8; oleic, 34.0; myristic, 0.1; palmitic, 8.7; stearic, 18.2; and arachidic, 5.8%. The unsaponifiable matter (1.42%) consisted mostly of sitosterol. The pods without seeds contained β-sitosterol, α-amyrin, ursolic and oleononic acids. The flowers are used as a vegetable; they are slightly bitter and require thorough washing before use. The tender leaves, pods and seeds are also eaten. The leaves are eaten by cattle, sheep and goats. In South India, the plant is used for green-manuring in the rice fields. The leaves are a source of blue dye, indigo, called *Mysore pala indigo* (yield, 0.33-0.50%). The tree bears handsome clusters of white, jasmine-scented, star-shaped flowers in profusion, which are much esteemed by Hindus for offerings at the temples (Chopra *et al.*, 1958).

3.6. Chemical constituents of *Wrightia tinctoria*

Four uncommon sterols, desmosterol, clerosterol, 2, 4-methylene-25-methylcholesterol and 24-dehydropollinastanol, in addition to several common
Phytosterols have been isolated from seeds of *Wrightia tinctoria* (Akibisa *et al.*, 1988). Leaves indicated the presence of flavonoids, glycoflavones-iso-orientin and phenolic acids (Daniel and Sabnis, 1978; Daniel and Sabnis, 1982). The compound, wrightial, a new terpene and other phytoconstituents, which were cycloartenone and cycloeucalenol were isolated and identified by fractionation of the methanol extract of the immature seeds (Ramchandra *et al.*, 1993). The unsaponifiable matter extracted from the bark by extraction with petroleum ether was fractionated with methanol to obtain triterpenes like β-sitosterol and β-amyrin and also wrightiadione (Warrier *et al.*, 1996). Five flavonoid compounds, indigotin, indirubin, tryptanthrin, isatin and rutin were isolated and identified from the leaves (Muruganadam *et al.*, 2000).

### 3.7. Traditional and clinical uses of *Wrightia tinctoria*

*Wrightia tinctoria* plant is used in Ayurveda, Unani and Siddha medicines as an astringent, febrifuge and tonic (Tomar and Singh, 1990). In Siddha system of medicine, it is frequently used for psoriasis and other diseases like dysentery, diarrhoea and as antihaeorrhagic agent. Fresh leaves are pungent and are chewed for relief from toothache (Kirtikar and Basu, 1975). Oil emulsion of *W. tinctoria* pods is used to treat psoriasis and also have fungicidal activity against *Pityrosporum ovale* recovered from dandruff (Mitra *et al.*, 1998; Krishnamoorthy and Ranganathan, 2000; Reddy *et al.*, 2000). The leaf extract of this plant has been found to have analgesic, anti-inflammatory and antipyretic activities (Ghosh *et al.*, 1985). The plant is very useful in the treatment of abdominal pain, skin diseases and diarrhoea (Shah and Gopal, 1988; Singh *et al.*, 1980; Joshi *et al.*, 1980). It is also called as “Jaundice curative tree” in south India. The juice of the tender leaves is used effectively in jaundice. It has also been used as antidysenteric, antidiarrhoeal and antihaemorrhagic agent in Siddha system of medicine (Singh *et al.*, 2003).
3.8. Main chemical constituents of 777 oil with possible mechanism in management of psoriasis

3.8.1 Rutin

Rutin, a natural flavonoid which has antioxidant, anti-inflammatory, anticarcinogenic, antithrombic, cytoprotective and vasoprotective activities (Kuntic et al., 2007; Yang et al., 2008; Mauludin et al., 2009). The anti-inflammatory activity of rutin was investigated on carragenan-induced inflammation model. Intraperitoneal administration of rutin at a daily dose equivalent to 80 mg/kg showed the inhibition in both acute and chronic phases of inflammation. It was most active in chronic phase and was extremely effective in reducing edema, nodules and ankylosis (Guardia et al., 2001).

Oral administration of Rutin (100 mg/kg) reduced rat paw oedema starting 2 hours after carrageenan injection. It also reduced significantly, the polymorphonuclear neutrophils chemotaxis to formyl-methionyl-leucyl-phenylalanine (fMet-Leu-Phe) receptor in a dose-dependent manner. Moreover, elastase exocytosis, induced by both stimuli, was partially inhibited by rutin (Selloum et al., 2003).

Rutin inhibited IgE or phorbol-12-myristate 13-acetate and calcium ionophore (PMACI) mediated histamine release in RBL-2H3 cells and also inhibited elevation of intracellular calcium. The Rutin decreased gene expression and production of all the pro-inflammatory cytokines after PMACI stimulation of NF-kB/DNA binding, and NF-kB-dependent gene repoter assay. The anti-inflammatory activity of the natural polyphenoic flavonoid rutin can be used potentially against psoriasis through the down-regulation of mast cell activation (Park et al., 2008).

An investigation of rutin against septic arthritis due to Candida albicans, (a major etiological agent that causes fungal arthritis) was evauated. An emulsified mixture of C. albicans cell wall and complete Freund’s adjuvant was injected into mice via hind footpad route once a day, for three days to induce septic arthritis. After twenty-four hours of final injection, rutin was given to the mice (1 mg/dose/mouse) intraperitoneally every other day three times. Footpad-edema was measured after 17th day. The rutin treatment reduced approx 45% of the edema at the peak day (day 11) of septic arthritis and 6 days after the peak, there was an apporex 35% additional reduction of the edema. The anti-arthritis activity mediated by rutin inhibited the nitric oxide production from macrophages and T-cells proliferation (Han, 2009).
3.8.2 Indigotin or indigo and indirubin

Indigo naturalis is used in traditional Chinese medicine for various inflammatory diseases and dermatosis. Indigo naturalis, contains major ingredients, indirubin, indigo, and tryptanthrin showed efficacy in treating psoriasis where indirubin and indigo are structural isomers. The study explores the possible anti-inflammatory effects and mechanisms of indigo naturalis and its major ingredients, indirubin, indigo, and tryptanthrin in human neutrophils. The study result showed that these ingredients significantly inhibited the release of O$_2$ and elastase, in formyl-l-methionyl-l-leucyl-l-phenylalanine/cytochalasin B-activated (FMLP/CB-activated) human neutrophils in a concentration-dependent fashion (Lin et al., 2009).