Materials and Methods

1. Survey, collection and conservation of important medicinal plants in South India

South India is considered to be a vast repository of tropical medicinal plants. Nearly 95% of the medicinal plants regularly utilised by the manufactures of indigenous system of medicine are collected from the forests, which are distributed across diverse habitats in Western Ghats & Eastern Ghats together with the subsidiary hills. They provide raw materials for over 500 ayurvedic formulations in more than 800 manufacturing units in Kerala. The spurt in the requirement of ayurvedic drugs that was witnessed during the past decades has resulted in the over-exploitation of the precious forest resources, often endangering many of them. Now that the natural source of medicinal plants is dwindling and the requirement of raw materials for the drug industry is steeply increasing, there has come a need for producing at least a few of them by cultivation. This is essential for sustaining the indigenous systems of medicine especially ayurveda. First and foremost task in this programme will be the collection of the plants available in our forests, their identification, documentation and description of their properties and uses. With this objective in view a detailed survey was conducted in selected areas in South India, especially in the Western Ghats. A consolidated account of the surveys conducted and plants collected is given in table I. Extensive exploration was undertaken for locating and assessing the availability of these
plants. For detailed study leading to identification and nomenclature, twigs were collected with fully-grown leaves, complete inflorescence, flowers and fruits as far as possible. In certain cases repeated visits were made to the localities, for satisfactory collection of the materials. Collected samples were brought to the laboratory for detailed studies leading to their taxonomic and scientific identity. Propagules of these plants were conserved and grown in the herbal farm of Kerala Ayurveda Pharmacy Ltd. (KAPL) at Nedumbassery, Cochin for future reference and further research.

The plants collected from various regions were raised in nursery beds, earthen pots or in polythene covers filled with potting mixture. Potted plants were kept in shade houses. These lath houses were made with wood and metal pipe frames. The sides as well as the top were covered with agro pack shade nets, which provided 50% or 75% shade depending upon the requirement. Seeds were sown in seedbeds or pots to raise the seedlings. In some cases plants were raised from stem, root or leaf. Selected seedlings raised from various planting materials were cultivated in field to monitor their performance and to standardise the agro-techniques.

Preparation of herbaria

Plants collected and studied during the course of the investigation were made into herbaria for future reference and study. This preservation included wet as well as dry methods.

Angiosperm materials, collected were so chosen that it is perfect and complete for identification. For herbaceous plants, the whole plants were collected.
In case of large plants, small branches or leafy twigs, sufficient to fill a sheet were collected. As far as possible, the materials bearing flowers and fruits were collected. In the case of unisexual plants, care was taken to collect both female & male flowers or fruits. Large flowers were cut longitudinally to facilitate pressing and later mounting. Conifers and succulent plants were momentarily dipped in boiling water to facilitate quick drying. Aquatic plants were collected by slipping a sheet of paper under them in water and the sheets were lifted along with specimens. Collected specimens were tagged with field number and were kept in a vasculam in between sheets of thin paper/tissue paper.

Field notes were also prepared on habit, habitat, flower colour, locality, altitude and other interesting characteristics, which cannot be preserved in herbarium sheets. (Santapau 1955.a, 1955.b)

Large fleshy specimens were preserved immediately after collection in preserving fluid made up of formalin, glacial acetic acid & 50% alcohol in the ratio 5:5:90 (Mc Lean & Cook 1965).

The collections brought back from the field were placed between dry absorbent papers in drying presses. While transferring the specimens from the vasculam to the drying press, they were straightened and arranged so as to make them appear clearly on the mounting sheet. At least one leaf in each specimen was arranged with the under surface facing upwards to facilitate study of the structure of the leaf. The absorbent papers were changed daily for a few days. In case of wet weather, materials were dried partially with the help of an electrical drier before mounting.
Well dried specimens were glued on to herbarium sheets of standard size (42 x 29cm). Mounted specimens were neatly and scientifically labeled on the right hand side of the bottom corner of the herbarium sheets. They were scientifically arranged and were placed in thin paper folders. Insect repellents such as napthalene or paradichloro benzene were sprayed to protect from the attack of insects. All herbarium materials prepared during this study have been deposited in the KAPL herbarium, a herbarium maintained by the R & D Division of Kerala Ayurveda Pharmacy, Nedumbassery, Cochin.

2. **Performance appraisal of selected medicinal plants under Kerala conditions.**

Selected plants, which are not cultivated in Kerala on a wide scale but consumed in the state in large quantities in the manufacture of ayurvedic drugs, were introduced to the herbal farm of KAPL at Nedumbassery and were grown under experimental conditions in the field. The growth and yield pattern of the plants were recorded and specific observations were made regarding their suitability for cultivation, susceptibility to pests and diseases etc. The details of experimental conditions are given below.

2.1 *Aloe barbedensis* Mill.

Suckers of *Aloe* were collected from Salem, Tamil Nadu and raised in polythene bags. The plant was multiplied through suckers.

The experimental area was hoed well and channels of 30cm width were prepared at a spacing of 30 cm in plots of size 6m x 6m. Small pits were made in the channel at a spacing of 45cm and worked up with Farm Yard Manure @ 7 t/ha,
mussorie rock phosphate @ 2.5 t/ha and neem cake @ 1.25 t/ha cake. Suckers of A. barbadensis were planted in these pits and filled with topsoil.

Cow dung was applied at the rate of 2.5 t/ha at 3\textsuperscript{rd}, 6\textsuperscript{th}, 8\textsuperscript{th}, 10\textsuperscript{th}, 14\textsuperscript{th} & 18\textsuperscript{th} month after planting. It was applied in small lumps around the base of the plant during rainy months and in the form of slurry during summer season. Earthing up was done before the onset of monsoon to prevent water logging. Over crowding at the basal region was avoided by removing suckers.

2.2 \textit{Alpinia calcarata} Rosc.

Trial cultivation of \textit{Alpinia calcarata} was started during 1996 and the study was repeated during 1997 and 1998. Rhizomes of \textit{A. calcarata} were collected from Aromatic and Medicinal Plants Research Station, Odakkali, Ernakulam (Dist).

The experimental area was hoed well after mixing with farmyard manure @ 7 t/ha and Mussorie rock phosphate 2 t/ha. Four raised beds of size 9.6m x 4.5m x 45cm were made. Rhizome bits of size 5-7cm were planted in small pits made on the bed at a spacing of 60x60cm. Immediately after planting, the beds were mulched with dried leaves and irrigated. Rhizomes sprouted within 20 - 30 days of planting. After three months, cow dung in the form of slurry @ 5 t/ha and 17:17:17 complex fertiliser @ 750 kg/ha were applied, followed by earthing up of beds.

Plants were harvested by uprooting at an age of 18 months. After washing and cleaning, the rhizomes were cut into pieces of 10-12 cm size, dried well and weight recorded.
2.3. *Anisomeles malabarica* (Linn.) R.Br.ex.Sims

Seeds of *Anisomeles malabarica* were collected from Perunthara region of Tamilnadu in 1996. The seeds were soaked in water over night and were sown in seed beds of the size 2m x 2m. 250t/ha farmyard manure was applied basally. Immediately after sowing, the beds were irrigated and mulched with dry leaves. The seeds germinated in 10-15 days. Seedlings of 20–25 days age were transplanted to the main field.

<table>
<thead>
<tr>
<th>Plot size</th>
<th>40m²</th>
<th>Spacing</th>
<th>50cm x 50cm,</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total no. of plants</td>
<td>160</td>
<td>Manurai application : Basal application of FYM @ 250ton/ha.</td>
<td></td>
</tr>
</tbody>
</table>

An open field was selected for the cultivation of the plant. The land was worked up with FYM applied at 7t/ha. Four beds of size 2x5m were prepared and healthy seedlings were planted on beds at a spacing of 50x50cm in the middle May, 1996. Shade was provided during the initial one-month period.

The first harvest of leaves was done at an age of three months. Subsequent harvests were undertaken at the 5th, 7th, 10th, 13th, 16th, 20th and 24th months. Weeding and earthing up of soil along with top dressing of each harvest facilitated luxurient growth of plants. FYM @ 3t/ha was applied at the third 5th and 7th month. Plants were affected by pests or diseases. Observations on the yield of leaves in different harvests were recorded.

2.4 *Bacopa monnieri* (L.) Pennell.
Bacopa monnieri is one among the several drugs that should be used in the fresh form. But large-scale cultivation of the same in Kerala is seldom reported. The material available in local market is generally procured by local collectors from riversides, paddy fields etc. So it is likely to be contaminated with pesticide residues and heavy metals. Now a days there is an acute shortage of this material. Moreover, the marketed material is often found admixed with weeds. Taking all these into consideration cultivation of this drug plant was initiated. A trial was undertaken to evaluate the feasibility of cultivation of this medicinal plant. The experiments were conducted during 1997, 1998 and 1999.

Sample specimens of B. monnieri were collected from the wetlands around Fort Kochi of Ernakulam Dist. during 1997.

Being aquatic in nature, a waterlogged area under open sunlight was selected for the cultivation of this plant. The land was ploughed 3-4 times after applying farmyard manure @ 5 t/ha and neem cake and Mussorie rock phosphate, each at 2.5 t/ha. Plots of the size 20 x 2m size were prepared and stem cuttings were directly planted in the plots at a spacing of 5x5cm. Plants established very well within two weeks time. Water level was maintained at a height of 25-30 cm. After a period of 1½ month, an additional application of cow dung @ 2.5t/ha and 17:17:17 complex fertiliser @2.5 t/ha was undertaken.

First harvest was done after 3 months of planting and second at the 4½ months stage. Subsequent harvests were done at an interval of 45 days. The plants were cut 5 cm above soil level, washed, cleaned, dripped to dry and fresh weight recorded. After each harvest, cow dung was applied at 1.75 t/ha. As growth
proceeded, crop yield reduced substantially and after 1-year whole crop was harvested and replanted.

### 2.5 Holostemma adakodien Schultes.

Seedlings of *Holostemma adakodien* were collected from Ayyampuzha region of Ernakulam district. The seeds were sown in polythene bags filled with a 1:2:2 mixture of cow dung, red earth and river sand. Healthy seedlings were selected for transplanting in the field during second week of May, 1995. Soil was hoed well after a basal application of 6.25 t/ha farmyard manure, 2.5 t/ha neem cake and 1.25 t/ha Mussorie rock phosphate. Ten raised beds of size 3 m x 1 m x 45 cm were prepared at a spacing of 30 cm. Seedlings were transplanted into small pits of 30 cm x 30 cm x 30 cm size at a spacing of 30 cm x 30 cm. Artificial shade was provided and the seedlings were irrigated twice daily till the onset of monsoon. Cow dung @5 tons/ha and NPK 17:17:17 complex fertilizer @ 1.875 ton/ha were applied once in three months. During hot season, half the above dose only was applied, in the form of slurry. Weeding and earthing up of the beds was done once in a month during rainy season and in alternate months during hot season.

Plants established in field within 10 days and started vining in 30-40 days. The vines were trailed on a trellis made of coir rope maintained at a height of 2m. The *pandal* also helped to provide shade at the base of the plants.

### 2.6 Indigofera tinctoria Linn.

*Indigofera tinctoria* leaves are the major ingredient of the ayurvedic preparation, *Neelabhringadi thailam*. Juice extracted from fresh leaves is used in
this preparation. To ensure the timely availability of fresh leaves cultivation of this plant was initiated at KAPL.

The seeds of *I. tinctoria* were collected from Manjapra region in Ernakulam district. Seeds were separated from pods only at the time of planting. Viable seeds were selected by the flotation test and the seeds were soaked in water overnight to facilitate easy germination. Good seeds thus selected were broadcasted on raised beds during October 1996. Almost 80% of the seeds germinated within 20-25 days. Two plots of 40m² each were selected under open sun light and soil worked out with farmyard manure applied at 10 t/ha, neem cake at 2.5t/ha and Mussorie phosphate at 2.5 t/ha. Healthy seedlings were transplanted during January at a spacing of 50x50 cm. The plots were irrigated as and when required and shade was provided during the initial one month period. After establishment of the seedlings, shade was removed and plants were exposed to direct sunlight. Hoeing around the plants and earthing up were done periodically. Cow dung in the form of slurry at 5 t/ha and urea at 125 kg/ha were applied after each harvest.

First harvest of the leaves was done two months after planting. By the time leaves became bluish green in colour. Subsequent harvests were done at an interval of 45 days up to the age of 9 months and thereafter at an interval of 2 months. Roots were collected from the second plot from 8th month onwards up to the age of 24th month. The plants were uprooted carefully each time and the above ground portions were cut off. The roots were washed in running water and dried under shade before recording the weight. Severe pruning of the plants was found to accelerate leaf production. Delay in harvest of leaves was found to result in leaf fall.
2.7 *Kaempferia rotunda* Linn.

Sample rhizomes together with the tubers of *Kaempferia rotunda* were collected from Trichur market during January 1996. Small tubers were removed and the rhizomes were dipped in cow dung slurry, dried under shade, spread on sand and mulched with dried leaves.

Raised beds of size 3m x 1m x 45cm were prepared in a well drained garden soil in middle May, before the onset of monsoon. Soil was worked up after applying farmyard manure @ 10 t/ha, neem cake @ 2.5 t/ha and Mussorie rock phosphate @ 2.5 t/ha. Small pits were made at a spacing of 30 x 30 cm and the rhizome bits of 5-8 cm bearing 2 to 3 nodes were planted in these pits. Pits were covered with soil and the beds were mulched thoroughly with fresh leaves. The rhizomes started sprouting in a week’s time. Decaying green foliage that was used for mulching, formed a good manure for the developing plants. Farmyard manure @6.250 t/ha and fertilisers at the rate of 50 kg N, 50 kg P₂O₅ and 50 kg K₂O/ha were applied after the first and second weeding in the IInd & IVth month. Earthing up of the beds was done after each manure application.

The crop reached maturity in a period of 7 months when all the leaves dried off. The rhizomes were carefully dug out. After removing the roots and dried leaves, rhizomes were washed well, and the fresh weight of rhizomes were recorded.

2.8 *Operculina turpethum* (Linn.) Silva Manso

*Operculina turpethum* is not reported in Kerala either in wild or under cultivation. Since there is great demand for this drug in the market and the plant is
not available in Kerala, efforts were made to introduce the plant to Kerala and study its performance under cultivation.

Seeds of *Operculina turpethum* were collected from Jawaharlal Nehru Ayurvedic Medicinal Plants Garden and Herbarium, Poona in May, 1995. Seeds were sown in polythene bags filled with potting mixture. Seedlings at the 5-leaved stage were transplanted on a bed of size 1m x 1m x 60 cm after manuring with 1kg neem cake and 5 kg farmyard manure/bed. Irrigation was done on dry days. Farmyard manure at 10 kg/bed were applied and earthing up done at the 3\textsuperscript{rd}, 6\textsuperscript{th} and 10\textsuperscript{th} months. The plants flowered within a period of one year. Mature flowers were collected before the seeds got dispersed. The seeds were separated, dried and preserved for propagation in the next season.

2.9 *Pogostemon patchouli* Pellet

A field experiment was conducted in the research farm of KAPL, Nedumbassery during 1997 to estimate the yield potential of *Pogostemon patchouli*, Singapore variety under Kerala conditions. The soil of the area was tilled well and hoed with 5 t/ha neem cake and 20 t/ha farmyard manure. Beds of the size 4m x 1m x 50 cm were prepared 50 cm apart. One month old rooted cuttings were transplanted on the beds at a spacing of 30 x 30 cm. Each bed accommodated 39 plants and there were 6 beds. Shade was provided for 10 days till the cuttings were established. The plant tops consisting of 6-8 pairs of leaves were harvested when the colour of the leaves tended to turn pale. This corresponded with 45, 120, 180, 240 and 360 days after planting. After each cutting, cow dung slurry was applied at 25 t/ha and 20 kg each of N, P and K per hectare as 17:17:17 complex fertiliser. The fresh and dry weights of leaves harvested from each plot were recorded and the
yield of leaves per hectare was calculated. The growth of plants during the second year was very poor. The plants started to decline and were stunted in appearance. The experiment was repeated in another area during 1998.

3. Standardisation of agrotechniques of selected medicinal plants

Performance appraisal of selected medicinal plants introduced from various agroclimatic regions in South India helped to identify those which are well adapted to growth in the state. Studies were undertaken to work out optimum agronomic practices for commercial cultivation of some of these plants together with some exotic plants.

3.1 Growth and rhizome yield of *Acorus calamus* Linn. under different spacings and types of irrigation water used.

*Acorus calamus* is a sub aquatic plant well adapted to marshy soil and semi waterlogged conditions. An experiment was conducted during 1997 and repeated during 1998 to evaluate the effect of spacing and type of irrigation water on the growth and yield of *A. calamus*.

*Treatments: The treatments consisted of combinations of two spacings and two types of irrigation water*

Spacing : 30cm x 30cm, 60cm x 60cm

Types of irrigation water :

(a). Surface irrigation water. Rainwater collected

(b). Effluent water – water used for washing and cleaning raw drugs at KAPL raw drug division was collected and allowed to settle in an unlined tank. The clear water having BOD of approximately 900 mg/l was used for irrigation.
Plot size: 9m x 4.8m

The experiment was conducted in open paddy land. The soil was ploughed thrice and finally after adding farmyard manure at 10 t/ha and Mussorie rock phosphate at 5 t/ha. Sunken plots of 9m x 4.8 m were formed and seedlings of *A. calamus* were transplanted at a depth of 5 cm during June 1997. Plants established within 20 to 25 days. Major nutrients were supplied in the form of 17:17:17 complex fertilizer at the rate of 750 kg/ha in 2 split doses at 4<sup>th</sup> month and 8<sup>th</sup> month after planting. The plots were irrigated continuously to maintain standing water in the field. Hand weeding was done at the 2<sup>nd</sup> and 4<sup>th</sup> months.

Observations on the plant height and breadth of leaves were made on the 8<sup>th</sup> month. At the end of first year, the leaves started turning yellow and dry indicating maturity. Water in the field was drained off and the rhizomes were dug out carefully. The rhizomes were separated, cleaned properly and observations on length, diameter and weight were recorded.

### 3.2. *Adhatoda* spp.

Even though *Adhatoda beddomei* is regarded as pharmacologically more active than the other species, this plant is not cultivated on commercial scale and is generally procured from the wild source. Following great demand, the production of medicines that include *Adhatoda* has increased. At present *A. zeyalanica* is the species extensively used in medicine manufacture. There is need for the large-scale cultivation of this plant to ensure availability of genuine material for drug production. During field survey of medicinal plants, an interesting ecotype of *Adhatoda* was collected from Manjapra region of Ernakulam District in 1995. This
plant type is distinct from the other two in its habit. Taxonomic position of this plant could not be established due to lack of flowering.

3.2.1 **Histological studies in *Adhatoda* spp.**

Histological study of the roots and leaves of this new (*Manjapra*) ecotype was undertaken for comparative evaluation.
3.2.2 Comparative evaluation of types of *Adhatoda* (*A. zeyalanica, A. beddomei* & the *Manjapra* ecotype)

Since this ecotype is also used in ayurvedic medicine along with the other two species, a study has been conducted at KAPL farm to compare the growth rate and yield potential of the three types of *Adathoda*.

**Treatments**: 3 types of *Adhatoda* (*A. zeyalanica, A. beddomei* and *Manjapra* ecotype)

Nursery beds of the size 2m x 2m were prepared under 75% Agropack shade nets. Terminal stem cuttings of 10 to 15cm were planted to these beds at a spacing of 10cm x 10cm. Root initiated within 15 to 20 days. At the four leaved stage, the rooted cuttings were transplanted at a spacing of 30cm x 30cm on to beds of size 2.1m x 1.2 m x 30 cm prepared in the main field. There were three beds in each plot.

Farmyard manure at 5 tons/ha and neem cake at 2.5 tons/ha were applied to the soil prior to bed preparation. The plants branched profusely and there was luxuriant vegetative growth. Leaves were harvested after 3 months of planting and subsequent harvests were taken at an interval of 45 days. Each harvest was followed by application of 17:17: 17 complex fertiliser @ 750 t/ha. Observations on plant height and leaf dimensions were recorded three months after planting. Due to severe pruning of leaves, plants did not come to flowering. The yields declined after two years. At this stage, the leaf was harvested and weight recorded.

3.3 *Holostemma adakodien* Schultes
3.3.1. Biochemical changes in *Holostemma adakodien* during growth

Nursery beds of the size 2m x 2m x 45cm were prepared in well drained soil. Furrows of 5cm depth were prepared 20 cm apart. Root tuber cuttings of size 5 to 7cm were planted in these channels at a spacing of 15 cm and covered immediately with a layer of soil, irrigated and mulched with green leaves. The tubers sprouted within 15 - 20 days.

The main field was prepared as described under Section 2.5. Seedlings at the 4-leaf stage were transplanted to the beds at a spacing of 30 x 30cm. Shading, manuring and after care were done as described under Section 2.5.

Five plants each selected at random were uprooted carefully without damage of roots at different stages of growth and the dry weight was recorded in each case. A sub sample was subjected to analysis of total alkaloids, total carbohydrates and crude protein.

3.3.2. Comparative yield of seed propagated and vegetatively propagated plants of *Holostemma adakodien* Schultes.

*Holostemma adakodien* can be propagated through seeds as well as root tubers. This trial was intended to compare the performance of seed propagated plants with that of plants raised through tuber cuttings.

| Treatments | 2 (Seed propagated plants, Clones raised through root cuttings) |
| Plot size   | 3m x 1m |
| Spacing     | 30 x 30 cm |
No. of plants per plot : 30

Seedlings : Mature fruits were collected during January - February in 1997. They were tied intact to prevent the dispersal of seeds and were stored in airtight containers till sowing. Nursery beds of the size 2m X 2m x 30 cm were prepared. Soil was worked up with a 1:2:2 mixture of cow dung, red earth and river sand. At the time of planting, the seeds were separated from the follicle. They were mixed with sand to facilitate even distribution and sown on the beds. Immediately after sowing, beds were mulched with green leaves. Almost 80% of the seeds germinated within 25 days.

Clones : Clones were raised as described in Section 3.3.1.

Transplanting was done on the 45th day of sowing in the case of seedlings and nursery planting of tuber cuttings in case of clones. Training of plants and after care was done as described in Section 2.5.

Root tuber yield of plants was recorded at 11, 15, 17, 18 and 24 months of planting in the main field. Five plants each selected at random from each plot were carefully uprooted, washed free of impurities, dried under shade and the weight of tubers was recorded. Protein content of the tubers was estimated by standard procedure.

3.3.3 Effect of soil tilth on the tuberisation of Holostemma adakodien Schultes.

Holostemma adakodien is characterised by a peculiar root growth pattern. Although the plant produced 10-15 roots, only the main root underwent tuberisation. After attaining a particular length, the main root branched into two or three along the horizontal plane. Later, the main root and the branches started
thickening and developed into root tubers. It was generally observed that a lot of variation existed in the natural population as regards the length of the main root and pattern of tuberisation. It was greatly influenced by the degree of compaction of the soil. In order to ascertain the scope of increasing the yield of tubers by manipulation of soil compaction, a field experiment was conducted at the KAPL farm during 1997 to study the effect of soil tilth on the root characteristics and tuber yield of *H. adakodien*.

**Treatments:** 2;  
T1 - Tilled  
T2 - Untilled

- **Plot size:** 3m x 1m  
- **Spacing:** 30 x 30 cm  
- **No. of plants per plot:** 30

The experiment was conducted in a compact laterite soil. In case of tilled treatment, the soil was tilled well using spade and with the loose soil, raised bed of 60 cm height was prepared. In case of untilled treatment, no tillage was given and rooted cuttings were planted offering minimum disturbance to the soil.

Both the plots received a uniform application of farmyard manure @6 tons/ha, neem cake 2.5 tons/ha and Mussorie rock phosphate @ 1.25 tons/ha. Manures were incorporated into the soil around the base of the plants.

Training of plants and after care was done as described in Section 2.5. The field experiment was terminated 17 months after planting. The shoots were cut and the underground portions were excavated by carefully removing the soil. The roots and tubers were washed free of adhering soil particles. Total length of each root and
length of the tuber portion were measured. Tubers were separated and fresh weight recorded.

3.3.4. Effect of time of planting on the performance of *Holostemma adakodien* Schultes.

*Holostemma adakodien* is a perennial crop of 18-24 months duration. It is a shade loving plant and exposure to direct sunlight results in sun scorching of leaves. Though it exhibits stunted growth during the rainy season, the plant over-winters by entering a phase of physiological inactivity. During December-January, the leaves are shed and most of the vines die back. Fresh growth is put up in the spring. The plant is thus highly responsive to climatic factors. Hence proper selection of the season of planting is very important for the successful cultivation of this crop.

*H. adakodien* is a long duration crop. The tubers are highly priced and for the requirement of this crude drug, the industry depends solely on the tubers collected from the western ghats in the Palakkad and Malappuram districts of the state. Large fluctuation is experienced in the availability, mainly because the plant is ready for harvest only in a particular period of the year. In order to assure availability of good quality material it is desirable to cultivate this crop and harvest it at the proper stage. It is also desirable to stagger planting of the crop in different periods of the year so that round-the-year availability of the harvested produce can be assured. With this objective in view, a field experiment was laid out at the KAPL farm, Nedumbassery during 1997 to study the comparative growth and yield performance of *H. adakodien* planted in May and August.

**Treatments**

- **T1** : Planting in May
T2 : Planting in August

Plot size : 3 m x 1 m

Spacing : 30 x 30 cm

Plants per plot : 30

At the time of land preparation, farmyard manure @6 tons/ha, neem cake 2.5 tons/ha and Mussorie rock phosphate @ 1.25 tons/ha were applied uniformly in all the plots. Training of plants and after care was done as described in Section 2.5.

In case of T1, the nursery of root cuttings was prepared in March-April and the clones were transplanted to the main field in May. In case of T2, the nursery was raised in June-July and the clones were transplanted to the main field in August.

Number of surviving plants was recorded 4 months and 10 months after planting. The length and breadth of five randomly selected leaves from five observational plants at flowering stage were recorded and mean values worked out.

May 1996 planted crop (T1) was harvested in December 1997 and August 1997 planted crop (T2) was harvested in February 1998.

3.3.5. Variation in the quality of *Holostemma adakodien* Schultes. in market

The crude drug *adapathiyan* is an expensive material and the demand often exceeds the availability. Under the situation, it is very difficult to obtain genuine material in the market. Market *adapathiyan* is often admixed with immature tubers and roots as well as with the root and tubers of other plants. A lot of variation can be observed in the material available in different markets and in different seasons. An extensive market survey was conducted to compare the quality of *Holostemma adakodien* Schultes. tubers available in the major drug markets of Kerala. Samples
were collected from crude drug dealers in Palakkad, Trichur and Cochin and were analysed for various physical and biochemical characteristics.

The samples consisted of cut pieces of root tubers and portions of roots. Physical parameters like colour, length and breadth of the material was noted. Samples were also analysed for protein and alkaloid content.
3.4. *Indigofera tinctoria* Linn.

3.4.1. Effect of date of planting on the performance of *Indigofera tinctoria*

Plot size : 15 x 3 m
Spacing : 75 x 75 cm
Treatments : 2
T1 : January planted
T2 : May planted

In case of treatment 1, seeds were sown on nursery beds in October 1995 and the seedlings were transplanted to flat beds in the main field in January 1996. In treatment 2, nursery was raised in February 1996 and seedlings transplanted on to raised beds in May 1996. Artificial shade was provided for a period of two weeks after transplanting.

In both the cases, farmyard manure at 10 t/ha and neem cake at 2.5 t/ha were applied to the soil at the time of bed preparation. Cow dung at 2.5 t/ha and N at 75 kg/ha as urea were applied after each harvest. Top dressing was preceded by weeding and succeeded by earthing up. Irrigation was done as and when necessary.

Growth parameters of the plant like height and number of branches were recorded at the two-month and three-month stage.

January planted crop became ready for harvest within 3 months and subsequent harvests were done every 45 days. The growth in case of May planted crop was faster and hence the first harvest was done in 2 months and subsequent
harvests at 1-month intervals. Plant tops were cut at about 50 cm from ground level and weight recorded.

### 3.4.2 Effect of maceration of neelamari leaves on the extraction of colour by oil

The major process in the preparation of the ayurvedic drug neelabhringadi oil is the extraction of the pigments in neelamari leaves with oil. The leaves are boiled with coconut or sesamum oil for almost six hours to extract the colour and other factors. Maceration of the whole leaves prior to extraction with oil can be expected to increase the extraction of colour and related materials. On the other hand, since the oil extraction process involves prolonged boiling with oil, maceration of leaves may not have much influence on the extraction of colour. An experiment was conducted to study the effect of maceration on the extraction of colour from neelamari, separately with old and young leaves.

**Treatments:**

- **T1:** Old neelamari leaves
- **T2:** Young neelamari leaves
- **T3:** Juice of old neelamari leaves
- **T4:** Juice of young neelamari leaves

For extraction of neelamari leaves with oil, 7 g of the whole leaf material or its juice was put into 100 ml coconut oil taken in a glass beaker. The oil was boiled for about 10 minutes over a gentle flame on a bunsen burner to *manal pakam*. The contents were immediately strained through cotton cloth.

*Neelamari* leaves in T1 and T3 comprised of old leaves collected from the lowermost portions of branches. The leaves in T2 and T4 were collected from the growing tips of branches. In T1 and T2 the whole leaves were as such put into oil and boiled for extraction. In T3 and T4, the leaves were macerated using a mortar
and pestle with minimum quantity of water and the juice was expressed. The juice thus obtained was boiled with oil.

The colour of the oil extract was observed visually and scientifically recorded. The extract was scanned in a scanning uv spectrophotometer and the spectrum for 400-700 nm was recorded.

3.4.3 Effect of maturity of leaf on the oil extractable colour of neelamari

Fresh leaves of neelamari is an important ingredient of Neelabhringadi oil preparation. The preparation is characterised by a dark green colour and the quality of the product is often judged by its colour. In classical text Sararngadhara samhitha (Achutha warrier, 1955), it is prescribed that the fresh leaves of the plant should be extracted and used for the preparation. No specification is available regarding the age and maturity of the leaves to be used. In practice, the plant tops are harvested as a whole before flowering and all the leaves are used as such. Information on the influence of the age of the leaf on the quality of the product is not available. An experiment was conducted to study the influence of the maturity of the leaf on the colour intensity of neelabhringadi oil.

Treatments:

Leaves of four age groups can be identified on the neelamari plant. The oldest leaves are large in size with a bluish green lustre. The next group is slightly younger than the former and in comparison they have less bluish hue. The third group is almost green in colour. The fourth group consists of comparatively the youngest leaves, they are smaller in size and pale green in colour.

T1 : Juice of leaves from first group

T2 : Juice of leaves from second group
T3 : Juice of leaves from third group

T4 : Juice of leaves from fourth group

7 grams of leaf material was put into 100 ml coconut oil taken in a glass beaker. The oil was boiled for about 10 minutes over a gentle flame on a bunsen burner to *manal pakam*. The contents were immediately strained through cotton cloth. The colour of the oil extract was observed visually and recorded.

### 3.4.4 Effect of ingredients other than *neelamari* on the colour of *neelabhringadi* oil

The ingredients of *neelabhringadi* oil consist of *Eclipta alba* (*kayyunyam*), *Cardiospermum halicacabum* (*uzhinja*), *Bacopa monnieri* (*brahmi*), *Centella asiatica* (*kodavan*), *Emblica officinalis* (*nellickka*) and *Indigofera tinctoria* (*neelamari*). For the preparation of *neelabhringadi* oil, these materials are macerated with small quantity of water to express the juice. The juice is in turn extracted with mixture of coconut oil and castor oil. *Neelamari* extract is intensely bluish violet in colour. The oil extract of other components has a pale green colour. However, the final product has a characteristic dark green colour. This experiment was conducted to study the interaction between *neelamari* and other ingredients in producing the characteristic colour of *neelabhringadi* oil.

**Treatments**
- **T1** : *Oil extract of neelamari leaves*
- **T2** : Oil extract of other ingredients of *neelabhringadi* oil
- **T3** : Oil extract of *neelamari* leaves with other ingredients.

In T1, the juice extracted from 7 gm of fresh *neelamari* leaf was added to 100 ml coconut oil taken in a glass beaker. The oil was boiled for about 10 minutes
over a gentle flame of a bunsen burner to *manal pakam*. The contents were immediately strained through cotton cloth.

In T2, the juice of other ingredients of *neelabhringadi* oil viz., *Bacopa monnieri* (13g), *Eclipta alba* (20g), *Cardiospermum halicacabum* (20g), *Centella asiatica* (13g) and *Emblica officinalis* (10g) was prepared by macerating a mixture of the above ingredients with 20 ml water. The oil extract of juice was prepared as in T1.

In treatment T3, *neelamari* leaves as in T1 together with other ingredients as in T2 were mixed and the juice extracted. The oil extract of juice was prepared as in the previous cases.

The colour of the oil extracts were visually observed and noted. Absorbance at 670 nm, 600 nm and 580 nm were measured using a spectrophotometer. Absorption spectrum between 400 and 700 nm light was also recorded.

### 3.5 Shade requirement of *Mentha arvensis* Linn.

An experiment was conducted to compare the growth and yield performance of *Mentha arvensis* under open and shaded conditions. Seedlings collected from Dhanwantharivanam, Bangalore were brought to the medicinal garden of KAPL in 1996. Subsequent generations were raised under artificial shade to produce sufficient number of suckers.

Treatments : T1 : growing in open sunlight

T2 : growing under Agro Pack shade net of 50% shade

The field was prepared by hoeing several times. Cow dung at 30 t/ha and neem cake at 2.5 t/ha were added to the soil before forming plots of size 1m x 1m.
Suckers were transplanted at a spacing of 15cm x 15cm and replicated 5 times. All the plots were provided shade with coconut leaves for the initial 15-day period. After the establishment of the seedlings, the temporary shade in T1 was removed and 50% shade was provided in T2.

A 0.2% solution urea was sprayed on the foliage after 25 days of planting. Two-kg cow dung slurry and 20 g of 17:17:17 complex fertilizer per plot were applied after each harvest. Regular irrigation was given to keep the soil moist.

Height of the plant, length and breadth of leaf, number of branches per plant and internodal length were recorded on the 40th day from five randomly selected plants.

3.6 *Operculina turpethum* (Linn.) Silva Manso

3.6.1 Comparative yield of seed propagated and vegetatively propagated plants of *Operculina turpethum* (Linn.) Silva Manso

An experiment was conducted to determine the optimum stage of harvest and to compare the yield of seed and vegetatively propagated plants of *Operculina turpethum*. Seedlings were raised from seeds collected from the farm of KAPL. The field trial was laid out in May 1997. The experiment was repeated during 1998 to confirm the yield observations. Details of cultivation are presented in 2.8.

Treatments : 2

T1 : Seed propagated
T2 : vegetatively propagated

Plot size : 20 m x 2 m
Spacing : 1 m x 1 m
Vegetative propagation was done by compound layering. Runner shoots were covered with soil during the month of January for root initiation. The freshly produced sprouts were separated carefully along with roots and transplanted to the experimental plots.

Observations on biometric characters were recorded at the time of flowering from ten observation plants selected at random from each plot. Five plants were selected at random at each predetermined stage and yield observations recorded. These plants were uprooted, shoot and roots separated and the fresh weight was immediately noted. The harvested material was dried under partial shade for several days and dry weight recorded.

3.6.2 Nutrient management in *Operculina turpethum* Silva Manso

An experiment was conducted during 1997 to study the response of *O. turpethum* to organic and inorganic sources of plant nutrients. Details of land preparation are given in 2.8. A uniform basal application of farmyard manure @25 tons/ha and Mussorie rock phosphate @ 5 tons/ha was done before formation of bunds.

Plants raised through compound layering were transplanted at a spacing of 50 x 50 cm, on to beds of size 200 x 100 x 30 cm.

3.6.3 Physical characteristics of market *trivrit*

Collection of samples

Commercially available *trivrit* were collected from the following crude drug dealers.

a. SITCO, Mattancherry, Kochi, Kerala

b. Sarada Trading Co., Ludhiana, Punjab
c. Hindustan Herbs, Hyderabad, Andhra Pradesh

d. Sanjay Trading Company, Culcutta, West Bengal

Four samples, each weighing 200g were collected from each source. The samples were examined for colour, length and breadth of the pieces.

3.6.4 Biochemical evaluation of *Operculina turpethum* Silva Manso

Biochemical analysis of *Operculina turpethum*, the ayurvedic drug and *Marsdenia tenacissima*, its adulterant was undertaken to characterise the materials and to develop chemical methods for their identification. Analysis of representative samples of the following materials was undertaken.

1. *Operculina turpethum*, stem
2. *Operculina turpethum*, root
3. *Marsdenia tenacissima*, stem
4. *Marsdenia tenacissima*, root
5. *Trivrit* crude drug collected from market

Samples 1 to 4 were collected from authentic plants cultivated in the medicinal plants garden of KAPL, Nedumbassery, Cochin.
Processing of sample: The plant material was rinsed with distilled water and
dried under partial shade, powdered well in a mixer-blender and stored in glass
bottle until analysis.

Analysis of crude fibre: This was carried out following the procedure of
Maynard (1970). The sample was digested with 1.25% sulphuric acid and
subsequently with 1.25% sodium hydroxide. The residue was weighted and
incinerated. The crude fibre content of the sample was calculated from the loss in
weight upon incineration.

Extraction of the sample: The powdered sample was extracted separately
with one of the following solvents - chloroform, acetone or water. In the case of
organic solvents, 1 g material was soaked overnight in 20ml of the solvent. At the
end of the period, the extract was filtered through a plug of cotton wool. The
residue was washed 3 times with the solvent and washings collected and pooled.
The combined extract was evaporated to dryness over a water bath, cooled and
weight of extract determined.

To prepare the water extract, 1 g material was warmed on a hot plate with 30
ml water and the extract was filtered by passing through a plug of cotton wool. The
residue was washed 3 times with water and washings collected and pooled. The
combined extract was evaporated to dryness on a hot plate, cooled and weight of
extract determined. The material was re-dissolved in 1 ml of water and was
analysed by reversed phase TLC.

Reversed phase TLC analysis was carried out on 5 x 20cm TLC aluminium
sheets pre-coated with 0.2 mm RP-18 adsorbent (Merck Cat No.1.05560). Five μl
portion of each extract was spotted at the base of the plate using a graduated
capillary tube and developed with water + methanol(1:1). Spots developed on TLC analysis were visualised by exposing the plate to iodine vapour in an iodine chamber.

3.7 Comparative evaluation of *Piper* spp.

*Piper longum* is being widely cultivated in Kerala. However, no study is seen reported on the feasibility of cultivation of *Piper chaba a related species*. A study has been conducted to evaluate the performance of *P. chaba* in comparison with *P. longum*.

An experiment was conducted at the farm of KAPL to compare the growth, yield and biochemical characteristics of *Piper longum* and *Piper chaba during* 1995-97 and repeated during 1996-98.

Planting material of *P. chaba* was collected from Trippoonithura, Kerala (personal collection) and that of *P. longum* from Aromatic and Medicinal Plant Research Station, Odakali. Rooted cuttings of the plants were developed in polybags filled with a 2:2:1 mixture of red earth, river sand and powdered cow dung.

An area with well-drained soil was selected as the experimental site. The whole area was provided shade with 50% shade net. The area was tilled three times. *P. chaba* being a climber was trained on 2 m tall concrete standards erected at a spacing of 1x1 m. Four rooted cuttings were planted around each standard, at a spacing of 80x80 cm. Before planting, 10 kg FYM, 5 kg neemcake and 1kg Mussorie rock phosphate were incorporated in the soil around the standard. Rooted cuttings of *P. longum* were planted in soil prepared as above in plots of 1x1 m at a spacing of 50 x 50 cm. The plants were top dressed with FYM @ of 8 tons/ha and
50 g of 17:17:17 complex fertiliser per vine, two times a year. Plants were irrigated once a week.

Plants of *P.chaba* established within 90 days by producing climbing roots and started flowering about 150 days after planting. Flowers were produced at the leaf nodes. Spikes matured in 45 days when it became orange red in colour with maximum pungency. Mature fruits were hand picked at an interval of 15-20 days and air dried.

Vines of *P. longum* started flowering 3 months after planting. Flowers and fruits were produced almost throughout the year. On maturity, the fruits turned dark green. Mature fruits were hand picked at an interval of 15-20 days and air-dried.

Observations on the average length and breadth of leaf were recorded from five randomly selected plants on the 150th day. Similarly observations on the average length and diameter of fruits were recorded from five randomly selected plants at the time of harvest.

### 3.8 Performance of *Pogostemon patchouli* Pellet under shaded and unshaded conditions

Stem cuttings of Singapore variety patchouli were collected from a personal collection in Calicut in the year 1997. The species and variety were identified by comparison with authentic specimens maintained at the Kerala Agricultural University, College of Horticulture, Trichur.

Treatments: T1 : Unshaded

T2 : Shade provided by 75% sun pack agro shade net (Polypropylene/plastic net which allowed 25 % of sunlight to penetrate)
Soil was thoroughly worked up with sand to ensure a porous medium for the plants. Beds of the size 2 m x 1 m x 30 cm was prepared and mixed well with compost at the rate of 7.5 t/ha. Soft woodcuttings consisting of 4 young leaves and growing tip were planted at a spacing of 20x20 cm during May in 1997. There were 3 beds in each treatment and 50 plants per bed. The plots were irrigated daily. Observations on the number of established cuttings per plot were recorded after one month.

3.9 Response of *Ruta chalepensis* Linn. to shade

Specimen plants of *Ruta chalepensis* were collected from Dhanwantharivanam, Bangalore. Rooted cuttings of the plant were raised in polybags through soft woodcuttings.

Treatments: T1 : 75% sunpack agro shade net

T2 : unshaded

The land was prepared by repeated tillage and farmyard manure was applied at 10 t/ha. Rooted stem cuttings raised in polythene bags were transplanted to 1.8m x 3.6m plots at a spacing of 45cm x 45cm during October in 1995. The experiment was repeated for confirmatory results during 1996.

Number of plants established in the field was noted 45 days after transplanting. Plant height and number of branches per plant were recorded from five randomly selected plants in each treatment.
3.10 Performance of *Wedelia chinensis* (Osbeck) Merrill under different spacings.

An experiment was conducted during 1997 to evaluate the growth and yield of *Wedelia chinensis* under two spacings. Healthy terminal cuttings of *W. chinensis* of length 12-15 cm were collected from Govt. Ayurveda College, Thiruvananthapuram and were raised in nursery under shade. The plant was propagated through soft woodcuttings.

The experiment was conducted in a clayey water logged area under open sunlight. The soil was hoed well and FYM was applied at the rate of 12.5 t/ha.

Plot size: 3 m x 2 m

Treatments:
- T1 – Spacing of 25 x 25 cm
- T2 – Spacing of 50 x 50 cm

Rooted stem cuttings were transplanted to the plots during April-May. Plant tops were harvested at 60 days intervals and fresh weight recorded.

4. Phytochemical studies in Rasna

‘Rasna’ is an effective anti-rheumatic and anti-arthritic drug, which has wide application as a single drug as well as in compounded preparations. Charaka has included it in the “*Vayasthapana varga*”, the group of drugs that can maintain vigour and strength. Being “*Vathaharanam*”, which means the right choice for rheumatic complications, it is an indispensable component of almost all anti-rheumatic preparations in the form of *kwaths, arishtams, oils, kuzhambu* and *choornams*.

A clear identification of the plant source of *rasna* is not available in classical literature. Instead, an attempt is seen to describe the property of the plant which
includes tongue like leaf, fragrant roots, anti-rheumatic property, resemblance to
cardamom, high pungency etc. Plants which embody one or more of these
properties are traditionally used as rasna and they include *Pleuechea lanceolata* C.B
Clarke, *Vanda tessellata* Loud and Loud, *Alpinia galanga* Sw., *Acampe papillosa*
Lindl., *Saccolobium papillosum* Lindl., *Viscum album* Linn., *Withania coagulans*
Dunal., *Inula racemosa* Hook f., *Tylophora asthmatica* W & A and *Aristolochia
indica* Linn.

The selection of ‘rasna’ in different formulations is governed mainly by
commercial considerations and not on scientifically generated information. An
attempt was made to rank five of these raw drugs in the order of their anti-
rheumatic property based on the content of methyl cinnamate, an established anti-
rheumatic agent.

**Fluorescence studies:**

The Fluorescence exhibited by rasna drugs when exposed to ultra violet
light (254 nm) was studied in an effort to characterise them.

a. Powder as such: Finely powdered drug was spread uniformly on a glass
slide and viewed under ultra violet source (254 nm) and the Fluorescence
exhibited by the powder, if any was noted.

b. After impregnation of the powder with alcoholic alkali: Finely powdered
drug was impregnated with 1% solution of sodium hydroxide in alcohol and solvent
removed by evaporation. The resultant powder was spread on a glass plate and
kept under ultra violet light (254 nm) and the fluorescence exhibited by the
powder, if any was noted.
c. After impregnation of the powder with alcoholic alkali and mounting on nitrocellulose paper: The alkali impregnated powder prepared as under (b) was spread uniformly on nitrocellulose paper and viewed under ultra violet source (254 nm). The fluorescence exhibited by the powder, if any was noted.

**Preparation of kwath powder**

a. **Rasnerandadi kwath**

*Rasnerandadi kwath* powders were prepared separately using *Alpinia officinarum, Alpinia calcarata* and *Alpinia galanga* in place of rasna. Prescribed quantities of authentic materials were collected, cleaned, washed and dried separately. They were bulked and coarse powdered using a hammer mill fitted with 10 HP motor and were homogenised. The powder samples were kept in tightly stoppered glass bottles till analysis.

b. **Ashtavargam kwath** powder

*Ashtavargam kwath* powder samples were prepared using *Alpinia officinarum, Alpinia calcarata* and *Alpinia galanga* following the formulary given below

Prescribed quantities of authentic materials were collected, cleaned, washed and dried separately. They were bulked and coarse powdered using a hammer mill fitted with 10 HP motor and were homogenised. The powder samples were kept in tightly stoppered glass bottles till analysis.

c. **Rasnasapthakam kwath** powder

*Rasnasapthakam kwath* powder of the following composition was prepared.

Prescribed quantities of authentic materials were collected, cleaned, washed and dried separately. They were bulked and coarse powdered using a hammer mill
fitted with 10 HP motor and were homogenised. The powder samples were kept in tightly stoppered glass bottles till analysis.

**Preparation of kwath**

*Kwaths* were prepared from the *Rasnerandadi kwath* powders by extraction with water. One part of the powder was soaked in 16 parts of water and boiled continuously over a flame to reduce the volume to one eighth. The extract was strained and the residue was discarded. The *kwath* samples were immediately analysed.

**Estimation of methyl cinnamate**

a. Extraction:- The five raw drugs studied were carefully chopped into small pieces of about 1 cm size manually using a hand knife. 50g of the chopped sample was extracted in a soxhlet extractor using acetone for 2 – 3 days till the extract became colourless. The *kwath* powders were similarly extracted for 8 – 12 hrs.

The solvent in the extracts was removed under vacuum and the volume made unto 100 ml in a standard flask using HPLC grade water. The extracts were centrifuged at 8000 – 10,000 RPM for about half an hour and the clear supernatant analysed by HPLC.

The *kwaths* prepared also were centrifuged at 8000 – 10,000 RPM for about half an hour and the clear supernatant analysed by HPLC.

b. Estimation of methyl cinnamate: Methyl cinnamate content of the extracts of the crude drugs, *kwath* powders and *kwath* were estimated using high-pressure liquid chromatography (HPLC).
i. Equipment : Waters HPLC with L-7100 pump

ii. Detector : L-7420 UV- Vis Detector

iii. Wave length : 220 nm

iv. Column : C18, 4.8 x 25 cm

v. Mobile phase : Acetonitrile (5) + Water (95), degaussed before use

vi. Injection volume : 2 μl

vii. Calculation : The peak areas were determined by integration using a computer estimation was done by external standard method.

viii. Retention time of methyl cinnamate : 4.24 min

ix. Standardisation : 0, 5, 25, 50 and 100 μl of 100 ppm standard methyl cinnamate solutions prepared in acetonitrile were injected separately into the instrument and the corresponding areas under the peak of methyl cinnamate were noted. A graph was constructed with quantity of methyl cinnamate on x axis and peak area on y axis.

x. Estimation: Area under the methyl cinnamate peak in each sample was noted. The quantity of methyl cinnamate in the injection volume was determined by referring to the standard graph, prepared as in ix. The methyl cinnamate content of the sample was calculated from the data on the quantity of methyl cinnamate in the injection volume, quantity of sample analysed and the dilution factor.
RESULTS AND DISCUSSION