CHAPTER II

MATERIALS AND METHODS

Buds, flowers and fruits were collected from various places and fixed in Formalin-Acetic-Alcohol (F.A.A.), the composition of which is 90 cc of 70% alcohol, 5 cc of Formaldehyde and 5 cc of Glacial acetic acid.

Details about the source of material and collection are given below.

<table>
<thead>
<tr>
<th>Species investigated</th>
<th>Collected by</th>
<th>Locality</th>
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<tbody>
<tr>
<td><strong>Family: Aizoaceae</strong></td>
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<tr>
<td><em>Trianthema triquetra</em> Rottl. ex Wild.</td>
<td>Author S.K. University campus</td>
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<tr>
<td><strong>Family: Fabaceae</strong></td>
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<tr>
<td><em>Macropelium atropurpureum</em> (DC.) Urb.</td>
<td>Author S.K. University campus</td>
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<tr>
<td><strong>Family: Lamiaceae</strong></td>
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<tr>
<td><em>Coleus arboreus</em> Lour.</td>
<td>Dr. T. Pullaiah Anantapur town</td>
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<td>(=C. <em>aromaticus</em> Benth.)</td>
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Species identifications of the above materials were made with the help of Gamble's "Flora of Presidency of Madras" and other floras and later authenticated at Madras Herbarium, Coimbatore. Voucher specimens have been deposited in the Herbarium of Botany, Department of Sri Krishnaddevaraya University (SKU).

When the material floated in the P.A.A, a suction pump is used to remove the air so that they dip in the solution. Dehydration was carried in Tertiary butyl alcohol in gradual series of 70%, 90 and 100% alcohol for at least one day. Feeding was carried out near a 60 watt bulb and flakes of paraffin wax of 55°-60°C was added slowly. Feeding was done for about 6 hours from 10 A.M. to 4 P.M. Then the vial was kept in a oven which was maintained at 60°C. Next day the material was transferred to a petri dish containing molten wax. The material was kept in the petri dishes for 1-3 days depending upon the hardness of the material. Then the material was embedded in paraffin wax. Sections were cut at a thickness of 5-6 μm for Macroptelium atrumnurum, 4-6 μm for Coleus amboinicus, 3-5 μm for Trianthema trigonata depending on the stage of the development. The sections were stained in Delafield's haematoxylin and mounted with canada balsam. Diagrams were drawn with camera lucida.