4. Results

The results are explained under three headings:

4.1. Morphological characters of *C. ternatea*, *C. pluricaulis* and *E. alsinoides*

4.2. RAPD analysis

4.3. HPLC analysis

4.1. Morphological characters

Morphological characters represent the physical form and external structure of plants. This preliminary result was used to discriminate the source species of shankhpushpi. A comparison of morphological traits exhibited by *C. ternatea*, *C. pluricaulis* and *E. alsinoides* is given hereunder.

<table>
<thead>
<tr>
<th>Evaluated characters</th>
<th><em>Clitoria ternatea</em></th>
<th><em>Convolvulus pluricaulis</em></th>
<th><em>Evolvulus alsinoides</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Habit</td>
<td>Perennial herb</td>
<td>Perennial herb</td>
<td>Perennial herb</td>
</tr>
<tr>
<td>Habitat</td>
<td>Widely cultivated as ornamental plant</td>
<td>Open grassy fields, rocky soil along road sides in northern and central India and Bihar</td>
<td>Forest edges, scrub jungles and Sandy localities throughout India</td>
</tr>
<tr>
<td>Root shape</td>
<td>Upright, cylindrical, tortuous branched</td>
<td>Cylindrical, ribbed, light yellow</td>
<td>Elongated, cylindrical with lateral branches</td>
</tr>
<tr>
<td>Root texture</td>
<td>Hairy</td>
<td>Hairy</td>
<td>Hairy</td>
</tr>
<tr>
<td>Stem</td>
<td>Twining, slender, suberect at base</td>
<td>Prostrate or ascending, branching basally, Slender, cylindrical</td>
<td>Prostrate or ascending, slender, pubescent</td>
</tr>
<tr>
<td>--------------------------</td>
<td>-------------------------------------</td>
<td>-----------------------------------------------------------------</td>
<td>--------------------------------------------</td>
</tr>
<tr>
<td>Leaves (stipule)</td>
<td>Stipulate</td>
<td>Exstipulate</td>
<td>Exstipulate</td>
</tr>
<tr>
<td>Stipule shape</td>
<td>Narrowly triangular</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Petiole (present/absent)</td>
<td>Present</td>
<td>Sessile</td>
<td>Sub sessile</td>
</tr>
<tr>
<td>Leaf type</td>
<td>Compound</td>
<td>Simple</td>
<td>Simple</td>
</tr>
<tr>
<td>Shape of leaf/leaflet</td>
<td>Oblong or broadly ovate, slightly emarginate or obtuse</td>
<td>Linear to oblong, oblanceolate to lanceolate</td>
<td>Oblong, elliptic-oblong to lanceolate</td>
</tr>
<tr>
<td>Phyllotaxy of leaf</td>
<td>Opposite superposed</td>
<td>Alternate, distichous</td>
<td>Alternate, distichous</td>
</tr>
<tr>
<td>Venation</td>
<td>Reticulate unicostate</td>
<td>Reticulate unicostate</td>
<td>Reticulate unicostate</td>
</tr>
<tr>
<td>Inflorescence type</td>
<td>Solitary Axillary</td>
<td>1-3 flowers in axillary heads</td>
<td>Solitary Axillary</td>
</tr>
<tr>
<td>Flower bract</td>
<td>Linear</td>
<td>Linear to oblanceolate</td>
<td>Linear-subulate to linear –lanceolate</td>
</tr>
<tr>
<td>Bracteole</td>
<td>Suborbicular or obovate, membranous</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Flower based on sex organs</td>
<td>Monoecious</td>
<td>Monoecious</td>
<td>Monoecious</td>
</tr>
<tr>
<td>Flower shape</td>
<td>Papilionaceous</td>
<td>Infundibuliform</td>
<td>Infundibuliform</td>
</tr>
<tr>
<td>-------------------</td>
<td>----------------</td>
<td>-----------------</td>
<td>-----------------</td>
</tr>
<tr>
<td>Flower colour</td>
<td>Blue, white</td>
<td>White, light pink</td>
<td>Blue</td>
</tr>
<tr>
<td>Sepal shape</td>
<td>Infundibuliform</td>
<td>Infundibuliform</td>
<td>Infundibuliform</td>
</tr>
<tr>
<td>Sepals cohesion</td>
<td>Gamosepalous apex free</td>
<td>Polysepalous</td>
<td>Polysepalous</td>
</tr>
<tr>
<td>Sepals aestivation</td>
<td>Valvate</td>
<td>Quinquincial</td>
<td>Quinquincial</td>
</tr>
<tr>
<td>Corolla cohesion</td>
<td>Polypetalous</td>
<td>Gamopetalous</td>
<td>Gamopetalous</td>
</tr>
<tr>
<td>Corolla aestivation</td>
<td>Vexillary</td>
<td>Valvate</td>
<td>Valvate</td>
</tr>
<tr>
<td>Stamen number</td>
<td>10 (9+1) diadelphous</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Filament colour</td>
<td>White</td>
<td>White</td>
<td>White</td>
</tr>
<tr>
<td>Anther colour</td>
<td>Light yellow</td>
<td>White</td>
<td>White</td>
</tr>
<tr>
<td>Anther position</td>
<td>Diadelphous</td>
<td>Polyandrous</td>
<td>Polyandrous</td>
</tr>
<tr>
<td>Stamen adhesion</td>
<td>Posterior stamen free, and filaments of nine stamens are fused to form sheath around the ovary</td>
<td>Epipetalous</td>
<td>Epipetalous</td>
</tr>
<tr>
<td>Anther fixation</td>
<td>Basifixed</td>
<td>Basifixed</td>
<td>Basifixed</td>
</tr>
<tr>
<td>Ovary position</td>
<td>Perigynous</td>
<td>Hypogynous</td>
<td>Hypogynous</td>
</tr>
<tr>
<td>Carpel number</td>
<td>01</td>
<td>02</td>
<td>02</td>
</tr>
<tr>
<td>Locule number</td>
<td>01</td>
<td>02</td>
<td>02</td>
</tr>
</tbody>
</table>
### 4.2. RAPD analysis

The present study provides an optimization of primer screening for molecular characterization and evaluation of genetic relationship among the accessions of three plant species- *Clitoria ternatea*, *Convolvulus pluricaulis* and *Evolvulus alsinoides*, which are equated with the traditional Ayurvedic drug “Shankhpushpi”. The samples of plant species investigated were collected from different geographical localities in India.

Twenty five 11-mer primers (10 OPN- series and 15- G series) were screened to estimate genetic relationship among the different accessions through polymerase chain reaction (PCR). The primers of OPN- series produced relatively more amplification fragments as compared to primers of G- series. The primers of OPN- series produced maximum number of DNA fragments, ranging from 0.5-2.0 kb. Most of the amplifications were duplicated. Only bands that were constantly reproduced across amplifications were considered for the analysis. Bands with the same mobility were considered as identical fragments, receiving equal values regardless of their staining intensity.
4.2.1. Genetic diversity in *C. ternatea*

Genetic relationship among seventeen accessions of *C. ternatea* from different geographical regions of India was investigated by using randomly amplified polymorphic DNA (RAPD). Out of 25 primers screened, seven primers (OPN-01, OPN-02, OPN-03, OPN-04, OPN-06, OPN-09 and OPN-10) generated clear bands (Table 5). A total of 71 reproducible bands were generated, of which 32 were polymorphic. The percentage of polymorphism obtained was 45.07. Primer OPN-4 generated 75% polymorphic bands, followed by OPN-09 (60%) and OPN-01 (57.14%) (Fig. 4). The lower polymorphism was observed in the case of primers OPN-03 and OPN-06 (50% each) (Fig. 5) and OPN-10 (14.28%) (Fig. 6). The primer OPN-02 generated 100% monomorphic bands (Fig. 7) and hence can be used as marker for the authentication of *C. ternatea*. The number of RAPD bands was in the range of 7-14, with an average of 10 bands per primer. The level of polymorphism within the accessions of *C. ternatea* ranged from 0-0.75. The genetic similarity and genetic distance was in the range of 0.72-1.00 and 0.00-0.28, respectively (Tables 6, 7).

**Table 5. RAPD data and percentage of polymorphic bands in *C. ternatea***

<table>
<thead>
<tr>
<th>S.NO.</th>
<th>Primer code</th>
<th>No. of bands</th>
<th>Polymorphic bands</th>
<th>% Monomorphism</th>
<th>% Polymorphism</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>OPN-01</td>
<td>14</td>
<td>08</td>
<td>42.86</td>
<td>57.14</td>
</tr>
<tr>
<td>2</td>
<td>OPN-02</td>
<td>10</td>
<td>0</td>
<td>100.00</td>
<td>0.00</td>
</tr>
<tr>
<td>3</td>
<td>OPN-03</td>
<td>08</td>
<td>04</td>
<td>50.00</td>
<td>50.00</td>
</tr>
<tr>
<td>4</td>
<td>OPN-04</td>
<td>08</td>
<td>06</td>
<td>25.00</td>
<td>75.00</td>
</tr>
<tr>
<td>5</td>
<td>OPN-06</td>
<td>14</td>
<td>07</td>
<td>50.00</td>
<td>50.00</td>
</tr>
<tr>
<td>6</td>
<td>OPN-09</td>
<td>10</td>
<td>06</td>
<td>40.00</td>
<td>60.00</td>
</tr>
<tr>
<td>7</td>
<td>OPN-10</td>
<td>07</td>
<td>01</td>
<td>85.72</td>
<td>14.28</td>
</tr>
<tr>
<td>Average</td>
<td>71</td>
<td>32</td>
<td></td>
<td>54.93</td>
<td>45.07</td>
</tr>
</tbody>
</table>
Figure 4. RAPD fingerprint obtained with OPN-01 primer in different accessions of *C. ternatea*: M = Marker (λ DNA digested with Hind III and EcoR I), 1- *Clitoria* white Delhi, 2- *Clitoria* blue Delhi, 3- *Clitoria* white U. P., 4- *Clitoria* blue U. P., 5- *Clitoria* blue Haryana, 6, 7- *Clitoria* white M. P., 8, 9- *Clitoria* blue M. P., 10, 11- *Clitoria* white Tamil Nadu, 12, 13- *Clitoria* blue Tamil Nadu, 14, 15- *Clitoria* blue Udaipur, (Rajasthan), 16, 17- *Clitoria* blue Jodhpur (Rajasthan) (Arrows represent region specific bands).

Figure 5. RAPD fingerprint obtained with OPN-06 primer in different accessions of *C. ternatea*: M = Marker (λ DNA digested with Hind III and EcoR I), 1- *Clitoria* white Delhi, 2- *Clitoria* blue Delhi, 3- *Clitoria* white U. P., 4- *Clitoria* blue U. P., 5- *Clitoria* blue Haryana, 6, 7- *Clitoria* white M. P., 8, 9- *Clitoria* blue M. P., 10, 11- *Clitoria* white Tamil Nadu, 12, 13- *Clitoria* blue Tamil Nadu, 14, 15- *Clitoria* blue Udaipur, (Rajasthan), 16, 17- *Clitoria* blue Jodhpur (Rajasthan) (Arrows represent region specific bands).
Figure 6. RAPD fingerprint obtained with OPN-10 primer in different accessions of *C. ternatea*: M = Marker (λ DNA digested with Hind III and EcoR I), 1-*Clitoria* white Delhi, 2-*Clitoria* blue Delhi, 3-*Clitoria* white U. P., 4-*Clitoria* blue U. P., 5-*Clitoria* blue Haryana, 6, 7-*Clitoria* white M. P., 8, 9-*Clitoria* blue M. P., 10, 11-*Clitoria* white Tamil Nadu, 12, 13-*Clitoria* blue Tamil Nadu, 14, 15-*Clitoria* blue Udaipur, (Rajasthan), 16, 17-*Clitoria* blue Jodhpur (Rajasthan). Arrow represents region specific band.

Figure 7. RAPD fingerprint obtained with OPN-02 primers in different accessions of *C. ternatea*: M = Marker (λ DNA digested with Hind III and EcoR I), 1-*Clitoria* white Delhi, 2-*Clitoria* blue Delhi, 3-*Clitoria* white U. P., 4-*Clitoria* blue U. P., 5-*Clitoria* blue Haryana, 6, 7-*Clitoria* white M. P., 8, 9-*Clitoria* blue M. P., 10, 11-*Clitoria* white Tamil Nadu, 12, 13-*Clitoria* blue Tamil Nadu, 14, 15-*Clitoria* blue Udaipur, (Rajasthan), 16, 17-*Clitoria* blue Jodhpur (Rajasthan). Circle represents monomorphic bands in the genotypes of *Clitoria ternatea*. Hence can be used for the authentication of this plant.
Table 6. Average genetic similarity for seven primers in different accessions of *C. ternatea*

<table>
<thead>
<tr>
<th>Accession</th>
<th>01</th>
<th>02</th>
<th>03</th>
<th>04</th>
<th>05</th>
<th>06</th>
<th>07</th>
<th>08</th>
<th>09</th>
<th>10</th>
<th>11</th>
</tr>
</thead>
<tbody>
<tr>
<td>Del. white 01</td>
<td>-</td>
<td>1.00</td>
<td>1.00</td>
<td>0.97</td>
<td>0.97</td>
<td>0.74</td>
<td>0.80</td>
<td>0.81</td>
<td>0.77</td>
<td>0.77</td>
<td>0.77</td>
</tr>
<tr>
<td>Del. blue 02</td>
<td>1.00</td>
<td>-</td>
<td>1.00</td>
<td>0.97</td>
<td>0.98</td>
<td>0.72</td>
<td>0.80</td>
<td>0.80</td>
<td>0.78</td>
<td>0.78</td>
<td>0.78</td>
</tr>
<tr>
<td>U. P. white 03</td>
<td>1.00</td>
<td>1.00</td>
<td>-</td>
<td>0.97</td>
<td>0.97</td>
<td>0.72</td>
<td>0.79</td>
<td>0.80</td>
<td>0.78</td>
<td>0.78</td>
<td>0.78</td>
</tr>
<tr>
<td>U. P. blue 04</td>
<td>0.97</td>
<td>0.97</td>
<td>0.97</td>
<td>-</td>
<td>1.00</td>
<td>0.72</td>
<td>0.78</td>
<td>0.80</td>
<td>0.77</td>
<td>0.77</td>
<td>0.77</td>
</tr>
<tr>
<td>Har. blue 05</td>
<td>0.97</td>
<td>0.98</td>
<td>0.97</td>
<td>1.00</td>
<td>-</td>
<td>0.72</td>
<td>0.78</td>
<td>0.80</td>
<td>0.77</td>
<td>0.78</td>
<td>0.78</td>
</tr>
<tr>
<td>M. P. white 06</td>
<td>0.74</td>
<td>0.72</td>
<td>0.72</td>
<td>0.72</td>
<td>0.72</td>
<td>-</td>
<td>0.94</td>
<td>0.91</td>
<td>0.94</td>
<td>0.94</td>
<td>0.94</td>
</tr>
<tr>
<td>M. P. blue 07</td>
<td>0.80</td>
<td>0.80</td>
<td>0.79</td>
<td>0.78</td>
<td>0.78</td>
<td>0.94</td>
<td>-</td>
<td>0.98</td>
<td>0.94</td>
<td>0.98</td>
<td>0.98</td>
</tr>
<tr>
<td>T.N. white 08</td>
<td>0.81</td>
<td>0.80</td>
<td>0.80</td>
<td>0.80</td>
<td>0.80</td>
<td>0.91</td>
<td>0.98</td>
<td>-</td>
<td>0.97</td>
<td>0.97</td>
<td>0.97</td>
</tr>
<tr>
<td>T.N. blue 09</td>
<td>0.77</td>
<td>0.78</td>
<td>0.78</td>
<td>0.77</td>
<td>0.77</td>
<td>0.94</td>
<td>0.94</td>
<td>0.97</td>
<td>-</td>
<td>0.97</td>
<td>0.97</td>
</tr>
<tr>
<td>Rj. 1. blue 10</td>
<td>0.77</td>
<td>0.78</td>
<td>0.78</td>
<td>0.77</td>
<td>0.78</td>
<td>0.94</td>
<td>0.98</td>
<td>0.97</td>
<td>0.97</td>
<td>-</td>
<td>1.00</td>
</tr>
<tr>
<td>Rj. 2. blue 11</td>
<td>0.77</td>
<td>0.78</td>
<td>0.78</td>
<td>0.77</td>
<td>0.78</td>
<td>0.94</td>
<td>0.98</td>
<td>0.97</td>
<td>0.97</td>
<td>1.00</td>
<td>-</td>
</tr>
</tbody>
</table>

Del = Delhi (Hamdard Campus); U. P. = Uttar Pradesh (Lucknow); Har. = Haryana (Arjun Herbal Park- Kurukshetra); M. P. = Madhya Pradesh (Bhopal); T. N. = Tamil Nadu (Coimbatore); Rj. 1. = Rajasthan (Udaipur); Rj. 2. = Rajasthan (Jaipur).
Table 7. Average genetic distances for seven primers in different accessions of *C. ternatea*

<table>
<thead>
<tr>
<th>Accession</th>
<th>01</th>
<th>02</th>
<th>03</th>
<th>04</th>
<th>05</th>
<th>06</th>
<th>07</th>
<th>08</th>
<th>09</th>
<th>10</th>
<th>11</th>
</tr>
</thead>
<tbody>
<tr>
<td>Del. white 01</td>
<td>-</td>
<td>0.00</td>
<td>0.00</td>
<td>0.03</td>
<td>0.03</td>
<td>0.26</td>
<td>0.20</td>
<td>0.19</td>
<td>0.23</td>
<td>0.23</td>
<td>0.23</td>
</tr>
<tr>
<td>Del. blue 02</td>
<td>0.00</td>
<td>-</td>
<td>0.00</td>
<td>0.03</td>
<td>0.02</td>
<td>0.28</td>
<td>0.20</td>
<td>0.20</td>
<td>0.22</td>
<td>0.22</td>
<td>0.22</td>
</tr>
<tr>
<td>U. P. white 03</td>
<td>0.00</td>
<td>0.00</td>
<td>-</td>
<td>0.03</td>
<td>0.03</td>
<td>0.28</td>
<td>0.21</td>
<td>0.20</td>
<td>0.22</td>
<td>0.22</td>
<td>0.22</td>
</tr>
<tr>
<td>U. P. blue 04</td>
<td>0.03</td>
<td>0.03</td>
<td>0.03</td>
<td>-</td>
<td>0.00</td>
<td>0.28</td>
<td>0.22</td>
<td>0.02</td>
<td>0.23</td>
<td>0.23</td>
<td>0.23</td>
</tr>
<tr>
<td>Har. blue 05</td>
<td>0.03</td>
<td>0.02</td>
<td>0.03</td>
<td>0.000</td>
<td>-</td>
<td>0.28</td>
<td>0.22</td>
<td>0.20</td>
<td>0.23</td>
<td>0.22</td>
<td>0.22</td>
</tr>
<tr>
<td>M. P. white 06</td>
<td>0.26</td>
<td>0.28</td>
<td>0.28</td>
<td>0.28</td>
<td>0.28</td>
<td>-</td>
<td>0.06</td>
<td>0.09</td>
<td>0.06</td>
<td>0.06</td>
<td>0.06</td>
</tr>
<tr>
<td>M. P. blue 07</td>
<td>0.20</td>
<td>0.20</td>
<td>0.21</td>
<td>0.22</td>
<td>0.22</td>
<td>0.06</td>
<td>-</td>
<td>0.02</td>
<td>0.06</td>
<td>0.02</td>
<td>0.02</td>
</tr>
<tr>
<td>T.N. white 08</td>
<td>0.19</td>
<td>0.20</td>
<td>0.20</td>
<td>0.20</td>
<td>0.20</td>
<td>0.09</td>
<td>0.02</td>
<td>-</td>
<td>0.03</td>
<td>0.03</td>
<td>0.03</td>
</tr>
<tr>
<td>T.N. blue 09</td>
<td>0.77</td>
<td>0.78</td>
<td>0.78</td>
<td>0.77</td>
<td>0.77</td>
<td>0.94</td>
<td>0.94</td>
<td>0.97</td>
<td>-</td>
<td>0.97</td>
<td>0.97</td>
</tr>
<tr>
<td>Rj. 1. blue 10</td>
<td>0.23</td>
<td>0.22</td>
<td>0.22</td>
<td>0.23</td>
<td>0.22</td>
<td>0.06</td>
<td>0.02</td>
<td>0.03</td>
<td>0.03</td>
<td>-</td>
<td>0.00</td>
</tr>
<tr>
<td>Rj. 2. blue 11</td>
<td>0.23</td>
<td>0.22</td>
<td>0.22</td>
<td>0.23</td>
<td>0.22</td>
<td>0.06</td>
<td>0.02</td>
<td>0.03</td>
<td>0.03</td>
<td>0.00</td>
<td>-</td>
</tr>
</tbody>
</table>

Del = Delhi (Hamdard Campus); U. P. = Uttar Pradesh (Lucknow); Har = Haryana (Arjun Herbal Park- Kurukshetra); M. P. = Madhya Pradesh (Bhopal); T. N. = Tamil Nadu (Coimbatore); Rj. 1. = Rajasthan (Udaipur); Rj. 2. = Rajasthan (Jaipur).
4.2.2. Data analysis and construction of dendrogram

Based on the degree of divergence, 17 accessions of *C. ternatea* were grouped into two clusters, cluster I and cluster II, such that the accessions within the cluster have lesser divergence values than those between clusters. The first cluster contains accessions collected from Delhi, Kurukshetra (Haryana) and Lucknow (U. P.). The second cluster comprised accessions of Jodhpur and Udaipur (Rajasthan), Bhopal (M. P.) and Coimbatore (Tamil Nadu) (Fig. 8)

![Dendrogram](image)


4.2.3. Genetic diversity of *C. pluricaulis*

In order to study the diversity at DNA level in *Convolvulus pluricaulis*, twenty five primers were used for RAPD analysis. Although eleven primers amplified *Convolvulus*
DNA, only five primers responded by producing clear and reproducible banding patterns. Various banding patterns were revealed by different primers with the size ranging from 0.5-2.0 kb. A minimum of six (OPN-04 and G-01) and a maximum of eleven (OPN-01) unambiguously amplified bands were generated, furnishing a total of 37 bands (Table 8) with an average of 7.4 bands per primer and the number of polymorphic bands per primer ranged from 1-09 with an average of 4.8 polymorphic bands per primer. The primers OPN-04, OPN-01 and OPN-02 generated highest percentage polymorphisms of 100, 81 and 71.42 respectively (Figs. 9, 10), and the lower polymorphisms (16.66% and 14.28%) were obtained by G-01 and OPN-09 primers (Figs. 11, 12). Of the total bands generated, 15 bands were found to be monomorphic across all the accessions. The genetic similarity and distance was in the range of 0.66-0.93 and 0.07-0.34, respectively (Tables 9, 10). The average polymorphism among different accessions of *C. pluricaulis* ranged from 0.14-1.0.

**Table 8: RAPD data and percentage of polymorphic bands in *C. pluricaulis***

<table>
<thead>
<tr>
<th>S.NO.</th>
<th>Primer code</th>
<th>Total no. of bands</th>
<th>Polymorphic bands</th>
<th>% Monomorphism</th>
<th>% Polymorphism</th>
</tr>
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<tbody>
<tr>
<td>1</td>
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<td>11</td>
<td>09</td>
<td>18.82</td>
<td>81.18</td>
</tr>
<tr>
<td>2</td>
<td>OPN-02</td>
<td>07</td>
<td>05</td>
<td>28.58</td>
<td>71.42</td>
</tr>
<tr>
<td>3</td>
<td>OPN-04</td>
<td>06</td>
<td>06</td>
<td>0.00</td>
<td>100.00</td>
</tr>
<tr>
<td>4</td>
<td>OPN-09</td>
<td>07</td>
<td>01</td>
<td>85.72</td>
<td>14.28</td>
</tr>
<tr>
<td>5</td>
<td>G-01</td>
<td>06</td>
<td>01</td>
<td>83.34</td>
<td>16.66</td>
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<td>Average</td>
<td></td>
<td>37</td>
<td>22</td>
<td>40.55</td>
<td>59.45</td>
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</table>
Figure 9. RAPD fingerprint obtained with OPN-01 primer in different accessions of *C. pluricaulis*: M = Marker (λ DNA digested with Hind III and EcoR I), 1, 2 *C. pluricaulis* from Haryana (Kurukshetra Campus), 3, 4 *C. pluricaulis* from Haryana (Arjun Herbal Park- Kurukshetra), 5 *C. pluricaulis* from Jodhpur (Rajasthan), 6, 7 *C. pluricaulis* from U. P., 8-11 *C. pluricaulis* from Delhi, 12-15 *C. pluricaulis* from M. P., 16-18 *C. pluricaulis* from Udaipur (Rajasthan), 19-21 *C. pluricaulis* from Jaipur (Rajasthan). Arrows represent region specific bands.

Figure 10. RAPD fingerprint obtained with OPN-02 primer in different accessions of *C. pluricaulis*: M = Marker (λ DNA digested with Hind III and EcoR I), 1, 2 *C. pluricaulis* from Haryana (Kurukshetra Campus), 3, 4 *C. pluricaulis* from Haryana (Arjun Herbal Park Kurukshetra), 5 *C. pluricaulis* from Jodhpur (Rajasthan), 6, 7 *C. pluricaulis* from U. P., 8-11 *C. pluricaulis* from Delhi, 12-15 *C. pluricaulis* from M. P., 16-18 *C. pluricaulis* from Udaipur (Rajasthan), 19-21 *C. pluricaulis* from Jaipur (Rajasthan). Arrows represent region specific bands.
Figure 11. RAPD fingerprint obtained with G-01 primer in different accessions of *C. pluricaulis*: M = Marker (λ DNA digested with *Hind* III and *EcoR* I), 1, 2 *C. pluricaulis* from Haryana (Kurukshetra Campus), 3, 4 *C. pluricaulis* from Haryana (Arjun Herbal Park Kurukshetra), 5 *C. pluricaulis* from Jodhpur (Rajasthan), 6, 7 *C. pluricaulis* from U. P., 8-11 *C. pluricaulis* from Delhi, 12-15 *C. pluricaulis* from M. P., 16-18 *C. pluricaulis* from Udaipur (Rajasthan), 19-21 *C. pluricaulis* from Jaipur (Rajasthan). Circles represent region specific bands.

Table 9 Average genetic similarity for five primers in different accessions of *C. pluricaulis*
### Table 10 Average genetic distance for five primers in different accessions of *C. pluricaulis*

<table>
<thead>
<tr>
<th>Accession</th>
<th>01</th>
<th>02</th>
<th>03</th>
<th>04</th>
<th>05</th>
<th>06</th>
<th>07</th>
<th>08</th>
</tr>
</thead>
<tbody>
<tr>
<td>Con. Har. 01</td>
<td></td>
<td></td>
<td>0.73</td>
<td>0.86</td>
<td>0.91</td>
<td>0.92</td>
<td>0.77</td>
<td>0.82</td>
</tr>
<tr>
<td>Con. Har. 02</td>
<td>0.93</td>
<td></td>
<td>0.75</td>
<td>0.85</td>
<td>0.89</td>
<td>0.84</td>
<td>0.80</td>
<td>0.74</td>
</tr>
<tr>
<td>Con. Rj. I. 03</td>
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<td>0.66</td>
<td>0.71</td>
<td>0.69</td>
<td>0.70</td>
</tr>
<tr>
<td>Con. U. P. 04</td>
<td>0.86</td>
<td>0.85</td>
<td>0.70</td>
<td></td>
<td>0.83</td>
<td>0.84</td>
<td>0.86</td>
<td>0.80</td>
</tr>
<tr>
<td>Con. Del. 05</td>
<td>0.91</td>
<td>0.89</td>
<td>0.66</td>
<td>0.83</td>
<td></td>
<td>0.93</td>
<td>0.89</td>
<td>0.83</td>
</tr>
<tr>
<td>Con. M. P. 06</td>
<td>0.92</td>
<td>0.84</td>
<td>0.71</td>
<td>0.84</td>
<td>0.93</td>
<td></td>
<td>0.89</td>
<td>0.87</td>
</tr>
<tr>
<td>Con. Rj. 2. 07</td>
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<td>0.86</td>
</tr>
<tr>
<td>Con. Rj. 3. 08</td>
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<td>0.74</td>
<td>0.70</td>
<td>0.80</td>
<td>0.83</td>
<td>0.87</td>
<td>0.86</td>
<td></td>
</tr>
</tbody>
</table>

Har. 01 = Haryana (Kurukshetra Campus); Har. 02 = Haryana (Arjun Herbal Park-Kurukshetra); U. P. = Uttar Pradesh (Lucknow); Del. = Delhi (Hamdard Campus); M. P. = Madhya Pradesh (Bhopal); Rj. 1 = Rajasthan (Jodhpur); Rj. 2 = Rajasthan (Udaipur); Rj. 3 = Rajasthan (Jaipur).

4.2.4. Data analysis and construction of dendrogram
To analyze the genetic distance within different geographically located accessions of *C. pluricaulis*, the similarity coefficients were used to generate a tree for cluster analysis using the UPGMA method. The resulting dendrogram differentiated two major clusters. One contains a solitary genotype collected from Jodhpur (Rajasthan). The second cluster contains the accessions collected from Kurukshetra (Haryana), Delhi, Bhopal (M. P.), Udaipur, Jaipur (Rajasthan) and Lucknow (U. P.) (Fig. 13).

**Figure 13. Dendrogram based on RAPD analysis for the estimation of genetic diversity in different accessions of *C. pluricaulis* collected from different locations.** C1-C4 = Kurukshetra (Haryana), C5 = Jodhpur (Rajasthan), C6, C7 = Lucknow (Uttar Pradesh), C8-C11 = Delhi, C12-C15 = Bhopal (Madhya Pradesh), C16-C18 = Udaipur (Rajasthan), C19-C21 = Jaipur (Rajasthan).

### 4.2.5. Genetic diversity of *E. alsinoides*

A total of 25 primers were screened to study genetic diversity in *E. alsinoides*. Out of these only five (Table 11) that showed reproducible results were chosen to amplify the 24 selected accessions. A total of 47 bands were amplified and the polymorphic bands were 39. The number of RAPD bands was in the range of 5-15 with an average of 9.4 bands per primer. The mean percentage of polymorphism was 82.97 and molecular size ranged from 0.5-2.0 Kb. Out of 47 bands, 8 bands were common in all the samples (3, 1, 1, 3 by primers...
OPN-01, OPN-04, OPN-05 and OPN-06 respectively) which reflected certain homology of the samples. The primer OPN-02 generated highest polymorphism of 100% (Fig. 14), followed by OPN-05, 87.50% (Fig. 15). 80% polymorphism was obtained through primers OPN-01 (Fig. 16) and OPN-04. The primer OPN-06 generated least polymorphism of 66.66% (Fig. 17). The value of genetic similarity and genetic distance ranged from 0.51-0.96 and 0.04-0.49, respectively (Table 12, 13). The level of polymorphism within the population of *E. alsinoides* ranged from 0.66-1.0.

### 4.2.6. Analysis of data and construction of dendrogram

A dendrogram constructed on the basis of the similarity matrix data by unweighted pair group method with average (UPGMA) cluster analysis separated all the 24 accessions in to two main clusters at 0.50 similarity coefficient. The samples of Sohna (Haryana), Srinagar (Uttarakhand) and Lucknow (U. P.) form the first major cluster. The second cluster was Delhi, Jodhpur, Udaipur (Rajasthan), Pachmarhi, Bhopal (M. P.) and Coimbatore (Tamil Nadu) (Fig. 18).

<table>
<thead>
<tr>
<th>S.NO.</th>
<th>Primer code</th>
<th>Total no. of bands</th>
<th>Polymorphic bands</th>
<th>% Monomorphism</th>
<th>% Polymorphism</th>
</tr>
</thead>
<tbody>
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<td>1</td>
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<td>100.00</td>
</tr>
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<td>OPN-04</td>
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<td>04</td>
<td>20.00</td>
<td>80.00</td>
</tr>
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<td>4</td>
<td>OPN-05</td>
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<td>07</td>
<td>12.50</td>
<td>87.50</td>
</tr>
<tr>
<td>5</td>
<td>OPN-06</td>
<td>09</td>
<td>06</td>
<td>33.34</td>
<td>66.66</td>
</tr>
<tr>
<td></td>
<td>Average</td>
<td>47</td>
<td>39</td>
<td>17.03</td>
<td>82.97</td>
</tr>
</tbody>
</table>
Figure 14: RAPD fingerprint obtained with OPN-02 primer in different accessions of *E. alsinoides*: M = Marker (λ DNA digested with Hind III and EcoR I), 1-3 *E. alsinoides* from Haryana, 4, 5 *E. alsinoides* from Uttarakhand, 6 *E. alsinoides* from U. P., 7-9 *E. alsinoides* from Delhi, 10-15 *E. alsinoides* from Rajasthan, 16-18 *E. alsinoides* from Tamil Nadu (BSI), 19-21 *E. alsinoides* from Tamil Nadu (Mother Cry Temple), 22-24 *E. alsinoides* from Pachmarhi (M. P.). Arrows and circles represent region specific bands.

Figure 15: RAPD fingerprint obtained with OPN-05 primer in different accessions of *E. alsinoides*: M = Marker (λ DNA digested with Hind III and EcoR I), 1-3 *E. alsinoides* from Delhi, 4-6 *E. alsinoides* from Rajasthan, 7-9 *E. alsinoides* from Tamil Nadu (BSI), 10-12 *E. alsinoides* from Tamil Nadu (Mother Cry Temple), 13-15 *E. alsinoides* from Pachmarhi (M. P.), 16-18 *E. alsinoides* from Bhopal (M. P.), 19-21 *E. alsinoides* from Haryana, 22, 23 *E. alsinoides* from Uttarakhand, 24 *E. alsinoides* from U. P. Arrows represent region specific bands.
Table 12. Average genetic similarity for five primers in different accessions of *E. alsinoides*

<table>
<thead>
<tr>
<th>Accession</th>
<th>01</th>
<th>02</th>
<th>03</th>
<th>04</th>
<th>05</th>
<th>06</th>
<th>07</th>
<th>08</th>
<th>09</th>
</tr>
</thead>
<tbody>
<tr>
<td>Evol. Har. 01</td>
<td>-</td>
<td>0.84</td>
<td>0.70</td>
<td>0.66</td>
<td>0.73</td>
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<td>0.67</td>
<td>0.66</td>
<td>0.66</td>
</tr>
<tr>
<td>Evol. U. K. 02</td>
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<td>-</td>
<td>0.78</td>
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<td>0.77</td>
<td>0.64</td>
<td>0.65</td>
<td>0.66</td>
<td>0.66</td>
</tr>
<tr>
<td>Evol. U. P. 03</td>
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<td>0.78</td>
<td>-</td>
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<td>0.65</td>
<td>0.72</td>
<td>0.66</td>
<td>0.68</td>
<td>0.68</td>
</tr>
<tr>
<td>Evol. Del. 04</td>
<td>0.66</td>
<td>0.54</td>
<td>0.51</td>
<td>-</td>
<td>0.72</td>
<td>0.68</td>
<td>0.66</td>
<td>0.73</td>
<td>0.73</td>
</tr>
<tr>
<td>Evol. Rj. 05</td>
<td>0.73</td>
<td>0.77</td>
<td>0.65</td>
<td>0.72</td>
<td>-</td>
<td>0.72</td>
<td>0.73</td>
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<td>0.81</td>
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<tr>
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<tr>
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<td>0.87</td>
<td>-</td>
<td>0.73</td>
<td>0.73</td>
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<td>0.66</td>
<td>0.68</td>
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<td>0.81</td>
<td>0.78</td>
<td>0.73</td>
<td>0.96</td>
<td>-</td>
</tr>
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</table>

Har = Haryana (Sohna); U. K. = Uttarakhand (Srinagar); U. P. = Uttar Pradesh (Lucknow); Del = Delhi (Hamdard Campus); Rj = Rajasthan (Jodhpur); T. N1. = Tamil Nadu (BSI-Coimbatore); T. N2. = Tamil Nadu (Mothe Cry Temple-Coimbatore); M. P. 1. = Madhya Pradesh (Bhopal); M. P. 2. = Madhya Pradesh (Pachmarhi).

Table 13. Average genetic distance for five primers in different accessions of *E. alsinoides*

<table>
<thead>
<tr>
<th>Accession</th>
<th>01</th>
<th>02</th>
<th>03</th>
<th>04</th>
<th>05</th>
<th>06</th>
<th>07</th>
<th>08</th>
<th>09</th>
</tr>
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<td>0.34</td>
<td>0.33</td>
<td>0.34</td>
<td>0.34</td>
</tr>
<tr>
<td>Evol. U. K. 02</td>
<td>0.16</td>
<td>-</td>
<td>0.22</td>
<td>0.46</td>
<td>0.23</td>
<td>0.36</td>
<td>0.35</td>
<td>0.34</td>
<td>0.34</td>
</tr>
<tr>
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<td>-</td>
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<td>0.32</td>
</tr>
<tr>
<td>Evol. Del. 04</td>
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<td>0.49</td>
<td>-</td>
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<td>0.32</td>
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<td>0.27</td>
<td>0.27</td>
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<td>Evol. Rj. 05</td>
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<td>-</td>
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<tr>
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<td>0.32</td>
<td>0.28</td>
<td>-</td>
<td>0.13</td>
<td>0.22</td>
<td>0.22</td>
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<td>0.13</td>
<td>-</td>
<td>0.27</td>
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</tr>
<tr>
<td>Evol. M. P. 1. 08</td>
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<td>0.04</td>
<td>-</td>
</tr>
</tbody>
</table>

Har = Haryana (Sohna); U. K. = Uttarakhand (Srinagar); U. P. = Uttar Pradesh (Lucknow); Del = Delhi (Hamdard Campus); Rj = Rajasthan (Jodhpur); T. N1. = Tamil Nadu (BSI-Coimbatore); T. N2. = Tamil Nadu (Mothe Cry Temple-Coimbatore); M. P. 1. = Madhya Pradesh (Bhopal); M. P. 2. = Madhya Pradesh (Pachmarhi).
Figure 16. RAPD fingerprint obtained with OPN-01 primer in different accessions of *E. alsinoides*: M = Marker (λ DNA digested with Hind III and Eco RI), 1-3 *E. alsinoides* from Delhi, 4-6 *E. alsinoides* from Rajasthan, 7-9 *E. alsinoides* from Tamil Nadu (BSI), 10-12 *E. alsinoides* from Tamil Nadu (Mother Cry Temple), 13-15 *E. alsinoides* from Pachmarhi (M. P.), 16-18 *E. alsinoides* from Bhopal (M. P.), 19-21 *E. alsinoides* from Haryana, 22, 23 *E. alsinoides* from Uttarakhand, 24 *E. alsinoides* from U. P. Circles represent region specific bands.

Figure 17. RAPD fingerprint obtained with OPN-06 primer in different accessions of *E. alsinoides*: M = Marker (λ DNA digested with Hind III and Eco RI), 1-3 *E. alsinoides* from Delhi, 4-6 *E. alsinoides* from Rajasthan, 7-9 *E. alsinoides* from Tamil Nadu (BSI), 10-12 *E. alsinoides* from Tamil Nadu (Mother Cry Temple), 13-15 *E. alsinoides* from Pachmarhi (M. P.), 16-18 *E. alsinoides* from Bhopal (M. P.), 19-21 *E. alsinoides* from Haryana, 22, 23 *E. alsinoides* from Uttarakhand, 24 *E. alsinoides* from Lucknow (Uttar Pradesh). Arrows and circle represent region specific bands.
Chapter 4

Results

Figure 18. Dendrogram based on RAPD analysis for the estimation of genetic diversity in different accessions of *E. alsinoides* collected from different locations. C1-C3 = Sonha (Haryana), C4, C5 = Srinager (Uttarakhand), C6 = Lucknow (Uttar Pradesh), C7-C9 = Delhi, C10-C12 = Judhpur (Rajasthan) C13-C18 = Coimbatore (Tamil Nadu) C19-C24 = Bhopal, Pachmarhi (Madhya Pradesh).

4.3. HPLC analysis (Chemical profiling)

The medicinal effectiveness of the plant species is related to the quantity of that marker compound. Plant species, strain and geographical origin can be distinguished using chemical fingerprinting. The chromatographic analysis employing High Performance Thin Layer Chromatography (HPTLC), High Performance Liquid Chromatography (HPLC), Gas Chromatography (GC) as well as Mass Spectroscopy (MS) and Nuclear Magnetic Resonance (NMR) are capable techniques to create chemical profile of active constituents of herbal material. The chemical constituents that have been isolated from the three plant species- *C. pluricaulis, E. alsinoides* and *C. ternatea*, investigated in the present study, are presented in (Table 14). Kaempferol, a natural flavonol is reported to be present in all three
plants and, therefore, the said metabolite was selected as a chemical marker and quantified it in different samples through HPLC. The results are presented in Table 15.

Table 14. Chemical constituents in the source plants of Shankhpushpi

<table>
<thead>
<tr>
<th>C. ternatea</th>
<th>C. pluricaulis</th>
<th>E. alsinoides</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kaempferol</td>
<td>Kaempferol</td>
<td>Kaempferol</td>
</tr>
<tr>
<td>Clitorin (kaempferol-3-O-(2,6-di-o-rhamnosyl) glucopyranoside</td>
<td>Convolvine</td>
<td>Evolvuline</td>
</tr>
<tr>
<td>kaempferol-3-monoglucoside</td>
<td>kaempferol 3-glucoside</td>
<td>kaempferol-7-O-β-glucopyranoside</td>
</tr>
<tr>
<td>kaempferol-3-O-rhamnosyl-(1→6)-galactoside</td>
<td>6-Methyl-7-hydroxy coumarin</td>
<td>kaempferol-3-O-β-lucopyranoside</td>
</tr>
<tr>
<td>kaempferol-3-O-rhamnosyl-(1→6)-glucoside</td>
<td>3,4 dihydroxy Cinnamic acid</td>
<td>quecetine-3-O-β-glucopyranoside, caffeic acid</td>
</tr>
<tr>
<td>kaempferol-3-O-rutinoside</td>
<td>n-octacosanol</td>
<td>6-methoxy-7-o-β-glucopyranoside coumarin</td>
</tr>
<tr>
<td>kaempferol-3-O-neohesperidoside</td>
<td>n-dotria contanol</td>
<td>2-C-methyl erythritol, triacontane</td>
</tr>
</tbody>
</table>

Table 15. Kaempferol concentration in different samples of plants

<table>
<thead>
<tr>
<th>S.No</th>
<th>Plant samples</th>
<th>Amount of kaempferol (mg/g dry wt) ± SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td><em>C. ternatea</em> (Blue flowered), New Delhi (Hamdard Campus)</td>
<td>10.115±0.003</td>
</tr>
<tr>
<td>2</td>
<td><em>C. ternatea</em> (White flowered), New Delhi (Hamdard Campus)</td>
<td>10.921±0.005</td>
</tr>
<tr>
<td>3</td>
<td><em>C. ternatea</em> (Blue Flowered), U. P. (Lucknow)</td>
<td>11.653±0.032</td>
</tr>
<tr>
<td>4</td>
<td><em>C. ternatea</em> (White Flowered), U. P. (Lucknow)</td>
<td>10.149±0.290</td>
</tr>
<tr>
<td>5</td>
<td><em>C. ternatea</em> (Blue Flowered), M. P. (Bhopal)</td>
<td>16.67±0.017</td>
</tr>
</tbody>
</table>
The concentration of kaempferol in these three plant species varies among themselves as well as with geographical conditions. The maximum concentration of kaempferol (20.018 ± 0.006 mg/gm dry wt) was noticed in C. ternatea (blue flowered) collected from Arjun Herbal Park, Kurukshetra (Haryana) and minimum concentration (0.026±0.007 mg/gm dry wt) was detected in E. alsinoides (M. P.). The concentration of kaempferol is much higher in C. ternatea as compared to C. pluricaulis and E. alsinoides (Figs. 20-28).
Figure 19. Chromatograms of Kaempferol (Standard).
Figure 20. Chromatograms showing kaempferol content of *C. ternatea*, *E. alsinoides* and *C. pluricaulis* collected from U. P.
Figure 21. Chromatograms showing kaempferol content of *C. ternatea*, *E. alsinoides* and *C. pluricaulis* collected from Delhi.
Figure 22. Chromatograms showing kaempferol content of *C. ternatea*, *E. alsinoides* and *C. pluricaulis* collected from M. P.
Figure 23. Chromatograms showing kaempferol content of *C. ternatea*, *E. alsinoides* and *C. pluricaulis* collected from Tamil Nadu.
Figure 24: Chromatograms showing kaempferol content of *C. ternatea* and *C. pluricaulis* collected from Rajasthan.
Figure 25. Chromatograms showing kaempferol content of *C. ternatea*, *E. alsinooides* and *C. pluricaulis* collected from Haryana.
**Figure 26.** Kaempferol concentration in different samples of *C. ternatea.*

1. *C. ternatea* blue (Delhi)
2. *C. ternatea* white (Delhi)
3. *C. ternatea* blue (Uttar Pradesh)
4. *C. ternatea* white (Uttar Pradesh)
5. *C. ternatea* blue (Madhya Pradesh)
6. *C. ternatea* white (Madhya Pradesh)
7. *C. ternatea* blue (Tamil Nadu)
8. *C. ternatea* white (Tamil Nadu)
9. *C. ternatea* blue (Haryana)
Figure 27. Kaempferol concentration in different samples of *C. pluricaulis*.

1. *C. pluricaulis* (Madhya Pradesh)
2. *C. pluricaulis* (Haryana)
3. *C. pluricaulis* (Uttar Pradesh)
4. *C. pluricaulis* (Rajasthan)
5. *C. pluricaulis* (Delhi)
Figure 28. Kaempferol concentration in different samples of *E. alsinoides*.

1. *E. alsinoides* (Delhi)
2. *E. alsinoides* (Haryana)
3. *E. alsinoides* (Uttarakhand)
4. *E. alsinoides* (Uttar Pradesh)
5. *E. alsinoides* Madhya Pradesh (Pachmarhi)
6. *E. alsinoides* Madhya Prudish (Bhopal)
7. *E. alsinoides* collected from Tamil Nadu
4.4. Authentication of market samples of Shankhapushpi

Samples of Shankhpushpi were procured from different crude drug markets to ascertain their identity and also to find out as to which of the three plant species equated with the drug Shankhpushpi is being sold in the different regions of the country. Twenty five primers (10 of OPN series and 15 of G series) were screened to check the authenticity of market samples. Only four primers (OPN-03, OPN-04 and OPN-05, and OPN-06) yielded amplification products that produced clear and reproducible bands, while the rest did not amplify the DNA or resulted in smear or faint bands. Each RAPD reaction was repeated thrice to check the authenticity of the results; these bands were used to distinguish authenticated, adulterated and spurious samples. RAPD profile shown by the plant species equated with Shankhpushpi (C. ternatea, C. pluricaulis and E. alsinoides) was compared with that of market samples. The market samples were loaded along with C. ternatea, C. pluricaulis and E. alsinoides. The RAPD profile of none of the market samples resembled with RAPD profile of authentic sample C. ternatea, which indicates that the collected samples were not of C. ternatea (Fig 29). The RAPD profile of the market samples collected from Gujarat and U. P. find resemblance with C. pluricaulis (Fig. 30, 31; lane 1, 6, 7). RAPD profile of market samples collected from Rajasthan and West Bengal finds match with that of E. alsinoides (Fig. 30, 31; lane 1, 4, 5). However, samples of Assam and Kerala did not match with any of the plants equated with Shankhapushpi.

![Figure 29: RAPD patterns of authentic and market samples generated by Primer OPN-3. M- Molecular marker (λ DNA restricted with Hind III & EcoR I), Lane 1- C. ternatea (Authentic sample), Lanes 2-7- Market samples (2- Assam, 3- Kerala, 4- Bengal, 5- Rajasthan, 6- Gujarat, 7- Uttar Pradesh).]
Figure 30: RAPD patterns of authentic and market samples generated by Primer OPN-4. M- Molecular marker (λ DNA restricted with Hind III & EcoR I), Lane 1- C. pluricaulis, E. alsinoides, C. ternatea (Authentic samples), Lanes 2-7- Market samples (2- Assam, 3- Kerala, 4- Bengal, 5- Rajasthan, 6- Gujarat, 7- Uttar Pradesh). Circles represent specie specific bands.

Figure 31: RAPD patterns of authentic and market samples generated by Primer OPN-6. M- Molecular marker (λ DNA restricted with Hind III & EcoR I), Lane 1- E. alsinoides, C. pluricaulis, C. ternatea (Authentic samples), Lanes 2-7- Market samples (2- Assam, 3- Kerala, 4- Bengal, 5- Rajasthan, 6- Gujarat, 7- Uttar Pradesh). Arrows and circles represent specie specific bands.
Table 16. Authentication of “Shankhpushpi” by RAPD markers using primer OPN-04

<table>
<thead>
<tr>
<th>No. of amplified bands in (authentic) field sample</th>
<th>No. Of consensus bands between <em>C. pluricaulis</em> (CP) and market samples</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>UP</td>
</tr>
<tr>
<td>08</td>
<td>08</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>No. of amplified bands in (authentic) field sample</th>
<th>No. Of consensus bands between <em>E. alsinoides</em> (EA) and market samples</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>UP</td>
</tr>
<tr>
<td>04</td>
<td>04</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>No. of amplified bands in (authentic) field sample</th>
<th>No. Of consensus bands between <em>C. ternatea</em> (CT) and market samples</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>UP</td>
</tr>
<tr>
<td>04</td>
<td>04</td>
</tr>
</tbody>
</table>
Table 17. Authentication of “Shankhpushpi” by RAPD markers using primer OPN-06

<table>
<thead>
<tr>
<th>No. of amplified bands in authentic sample</th>
<th>No. Of consensus bands between <em>C. pluricaulis</em> (CP) and market samples</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>UP</td>
</tr>
<tr>
<td>04</td>
<td>04</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>No. of amplified bands in authentic sample</th>
<th>No. Of consensus bands between <em>E. alsinoides</em> (EA) and market samples</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>UP</td>
</tr>
<tr>
<td>06</td>
<td>06</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>No. of amplified bands in authentic sample</th>
<th>No. Of consensus bands between <em>C. ternatea</em> (CT) and market samples</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>UP</td>
</tr>
<tr>
<td>09</td>
<td>09</td>
</tr>
</tbody>
</table>

4.5. AFLP profile of market samples

The authentication of market samples collected from Delhi, M. P. and Gujarat was also done through AFLP analysis using six primers (The experiments were carried out at Tata Energy Research Institute, New Delhi). These samples were loaded with field samples of *C. ternatea* (collected from Delhi, M. P. and Tamil Nadu), *E. alsinoides* (Delhi, M. P. and Tamil Nadu) and *C. pluricaulis* (Delhi and M. P.). When the AFLP profile of market samples and field samples were compared, no consensus band (s) was found among the
market samples and authentic sample of *C. ternatea* and *E. alsinoides*. This confirms that none of the market samples contained *C. ternatea* and *E. alsinoides*. However, the presence of 11, 5 and 3 consensus bands (Fig. 32) between market samples and field sample of *C. pluricaulis* confirms that market samples share identity with *C. pluricaulis*.

**Figure 32. AFLP profile of market samples:** M- Marker (Tomato DNA), a-f *C. ternatea*, g-j *E. alsinoides*, k, l *C. pluricaulis*, m-p market samples (1- Gujarat, 2- Bhopal, 3- Delhi, 4- Gujarat). Arrows represent species specific bands.