

## Chapter 3: Materials and Methodology

### 3.1 Survey

Field survey was carried out and the habitat of Asteraceae members were studied. Comparative analysis of diversity in different districts and habitats has been done. Visits were done frequently in different seasons for comparison of seasonal variations.

#### 3.1.1 Selection of sites for the field visits and Sample Collection

Five districts of south Gujarat i.e. Surat, Tapi, Navsari, Dang and Valsad were selected as Western Ghats starts from Tapi basin. After literature survey of local works and pilot surveys, the site for the field visits were selected.

Frequent field surveys were carried out for four years (2019 – 2023) to locate various species of Asteraceae members from different regions of south Gujarat. The identified areas were thoroughly investigated by proper observations and taking all the measures. The most important duration for Asteraceae collection is the flowering period and hence more than 100 tours were arranged in accordance with their flowering season and to study phenology same place were visited more than once in the interval of one month.

During field visits, the data like type of habitat, soil, ecosystem, associated species, habit of plant in different habitat etc. were observed. The species were photographed in their natural habitats by DSLR camera (Nikon 3400) and relevant data were noted. Then plants or twigs with flowers were collected and were stored air tight in zip lock bags. Collected specimens were brought down to the Phytodiversity laboratory for further studies.

Herbarium were prepared for the collected samples. The procedure of herbarium preparation is as follows.

#### 3.1.2 Distribution Study

After Survey, the distribution of Asteraceae species was classified as follows:

1. Common: Plant is occurring commonly in different places very frequent.
2. Occasional: Plant is occurring in different places but not very frequent.
3. Abundant: Found in few places but population is large.
4. Rare: Plant is occurring in few places and not common.

5. Restricted. Plant's restricted to specific place.

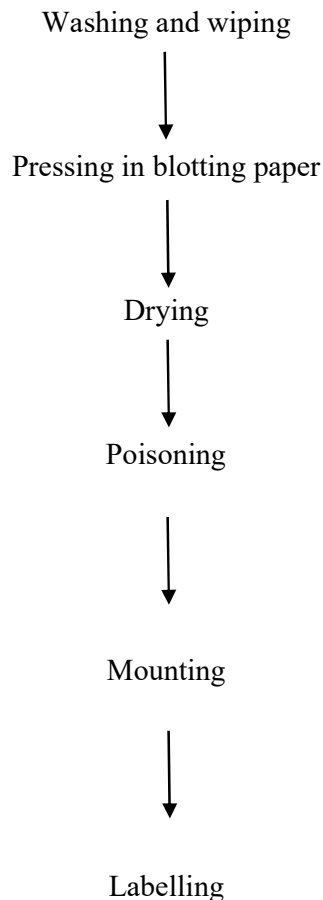
### 3.1.3 Sample Collection and Herbarium Preparation

After recording field observation,

Specimens were collected, dried, poisoned and mounted on herbarium sheets. The voucher specimens were deposited to BARO Herbarium.

Plant materials collected during field survey were used for the preparation of herbarium specimens. All the representative parts for identification of Asteraceae members were collected and used for herbaria preparation. Poisoning was done with an alcoholic solution of  $\text{HgCl}_2$  (0.1%). All the processed specimens were deposited at BARO, The Maharaja Sayajirao University of Baroda. The herbarium specimens were arranged alphabetically under each genus for the sake of convenience.

The procedure for herbaria preparation was done according to Jain and Rao (1977) which is as follows.



### 3.2 Morphological Studies and Plant Identification

Gross morphological studies were conducted at the field and laboratory. In field, macro characters like habit, stem, leaves, capitulescence, capitulum etc were studied and recorded. In laboratory micro characters like involucre, florets, anthers, styles, cypsela and pappus were studied under Leica stereomicroscope. These characters were noted down in the record book.

The Identification and confirmation of plant specimens were done using standard literatures (Hooker, 1883; Cooke, 1904; Shah, 1978; Hajra *et al.*; 1995; Yadav, 1979; Suryanarayan, 1968; Patel, 1971; More, 1972; Desai, 1976; Contractor, 1986; Mac, 1982; Reddy, 1987).

Bracketed Key have been prepared for easy identification of sub-families, tribes, genera and species. Plants of the world online (Royal Botanic Gardens, Kew) and Global Compositae Data (The International Compositae Alliance) have been followed for the species nomenclature. For each species, the correct botanical name followed by important synonyms has been cited.

The citation was followed by diagnostic description which includes habit, leaves, capitulescence, capitulum, florets, anthers, style, cypsela and pappus. Morphological description is followed by additional data like common name, vernacular name, specimen studied, phenology (Flowering and Fruiting), habitat, distribution and taxonomic notes.

### 3.3 Palynological Studies

Pollens were collected from the specimens which were collected from field. The procedure for processing pollen is as follows:

#### 3.3.1 Pollen collection and Storage

Fresh material was used for collection of pollens. The floret buds and open florets were collected from capitulum. They were kept in GAA (Glacial acetic acid) inside a 1.5 ml Eppendorf. Then they were crushed within the Eppendorf and were filtered with the help of muslin cloth to remove extra debris from sample. This process was repeated for 2-3 time to increases the yield of pollen. Then the Eppendorf were labelled and were stored.

### 3.3.2 Acetolysis

To study the pollen morphology properly, it is important to remove extra debris and cytological contents from pollen for which acetolysis of pollen was performed with modified Erdatmann's Acetolysis method (1960). The procedure is as follows.

The pollen was washed with glacial acetic acid for 2-3 time and then centrifuged at 3000 rpm for 5 mins. Then glacial acetic acid was decanted. Freshly prepared acetolysis solution (nine parts of Acetic Anhydride and one part of concentrated Sulphuric Acid) was poured in Eppendorf. The Eppendorf was then kept in water bath with temperature of 70° C for 5 mins. Then Eppendorf was taken out and was kept for centrifuge at 3000 rpm for 5 mins. The supernatant was then decanted and was of glacial acetic acid was given for 2-3 times. Then pollens were stored in 70% alcohol.

### 3.3.3 Slide Preparation

After acetolysis, semi-permanent slides of pollens were prepared. As mounting medium Kisser's glycerine jelly (7 g of gelatin, 21 mL of glycerin and 24.5 mL of distilled water) was used. A small piece of jelly was taken on the tip of needle and was stir in Eppendorf so that pollen stick to glycerine jelly. Then the piece of glycine jelly was kept on a clean slide. The slide was heated so that jelly melts (remove slide before bubbles starts to come out) and coverslip was kept on it. After cooling it, the coverslip was sealed with nail polish to avoid desiccation. Slide was labelled and kept in slide box.

### 3.3.4 Pollen Analysis

Pollens were analysed under compound light microscope (ESAW trinocular microscope) and confocal microscope (Carl Zeiss LSM 710). Light microscope was used for measurements which was taken in 400x magnification. For analysis of qualitative characters confocal microscope was used because the pollen wall has autofluorescence. The pollen was subjected to 488nm light. Z stack image for surface analysis and image of optical sections were taken which are used in preparation of photo plates.

### 3.4 Phyllogenetic Analysis

Morphological and Palynological data were used to prepared cladograms. The 56 characters of all 70 species with *Scaevola taccada* (Gaertn.) Roxb. (as it is the member of

Goodeniaceae family which is the sister group of Asteraceae) were arranged in tables. Codes for each character state of every characters were designated which is as follows:

### 3.4.1 Character Codes

1. **Habit-** Acaulescent (0), Decumbent (1), Diffuse (2), Erect (3), Liana (4), Shrub (5), Suffruticose (6).
2. **Branching-** Unbranched (0), Monopodial (1), Dichotomous (2).
3. **Phyllotaxy-** Rosette (0), Alternate (1), opposite (2).
4. **Leaf type-** Simple (0), Pinnately compound (1), Ternate (2).
5. **Leaf shape-** Linear (0), Lanceolate (1), Oblong (2), Ovate (3), Elliptical (4), Spathulate (5), Lyrate (6), Palmately compound (7).
6. **Margin-** Entire (0), Serrate (1), Dentate (2), Crenate (3), Spiny (4), Undulate (5), Lacerate (6).
7. **Apex-** Acute (0), Acuminate (1), Obtuse (2), Spiny (3).
8. **Base-** Amplexicaul (0), Attenuate (1), Auricled (2), Cordate (3), Cuneate (4), Decurrent (5), Obtuse (6), Truncate (7).
9. **Capitulescence-** Solitary (0), Raceme (1), Panicle (2), Spike (3), Cluster (4), Corymbose (5), Biparous (6).
10. **Head complexity-** Absent (0), Simple (1), Compound (2).
11. **Head type-** Absent (0), Ligulate (1), Discoid (2), Disciform (3) Radiate (4).
12. **Head sexuality-** Absent (0), Bisexual (1), Gynomonoeocious (2), Neutral and Bisexual (3), Unisexual (4).
13. **Head Bracteole-** Absent (0), Present (1).
14. **Receptacle-** Absent (0), Branched (1), Concave (2), Convex (3), Flat (4), Convex for Male and Concave for Female (5).
15. **Series of phyllaries-** Absent (0), Uniseriate (1), Biseriate (2), Multiseriate (3).
16. **Shape of Phyllaries-** Absent (0), Linear (1), lanceolate (2), Oblong (3), Ovate (4), Obovate (5).

17. **Apex of phyllaries-** Absent (0), Acute (1), Acuminate (2), Attenuate (3), Oblong (4), Obtuse (5), Spiny (6), Truncate (7).
18. **Palae-** Absent (0), Present (1).
19. **Type of Ray Floret-** Absent (0), Ligulate (1), Filiform (2) Bilabiate (3).
20. **Type of Disc Floret-** Absent (0), Ligulate (1), Tubular (2).
21. **Symmetry of Ray Floret-** Absent (0), Actinomorphic (1), Zygomorphic (2).
22. **Symmetry of Disc Floret-** Actinomorphic (0), Zygomorphic (1).
23. **Fertility of Ray Florets-** Absent (0), Fertile (1), Infertile (2).
24. **Fertility of Disc Florets-** Fertile (0), Infertile (1).
25. **Corolla lobes of Ray Floret-** 0 (0), 2 (1), 3 (2), 5 (3).
26. **Corolla lobes of Disc florets-** Absent (0), 4 (1), 5 (2).
27. **Texture of Corolla lobe-** Glabrous (0), Hairy (1).
28. **Number of anther lobes-** 3 (0), 5 (1).
29. **Anther apex-** Acute (0), Appendiculate (1), Attenuate (2), Obtuse (3), Truncate (4).
30. **Anther base-** Subentire or obtuse (0), Sagittate (1), Tailed (2).
31. **Style type-** Monomorphic (0), Dimorphic (1).
32. **Style Apex-** Acute (0), Attenuate (1), Obtuse (2), Subulate (3), Truncate (4).
33. **Fruit type-** Cypsela (0), Bur (1), Drupe (2).
34. **Cypsela type-** Monomorphic (0), Dimorphic (1).
35. **Cypsela shape-** Columnar (0), Fusiform (1), Gibbous (2), Obovate (3), Ovate (4), Spherical (5).
36. **Cypsela texture-** Glabrous (0), Hispid (1), Pubescent (2), Scrabid (3), Spiny (4), Tomentose (5).
37. **Cypsela base-** Without Disc (0), With Disc (1).

- 38. Pappus Type-** Absent (0), Bristles (1), Awns (2), Plumose (3), Ciliated (4), Scaly (5), Clubbed (6), Foliaceous (7), Setose (8).
- 39. Pappus series-** Absent (0), Uniseriate (1), Biseriate (2).
- 40. Pappus morphism-** Absent (0), Monomorphic (1), Dimorphic (2).
- 41. Pollen polarity-** Isopolar (0), Anisopolar (1).
- 42. Pollen symmetry-** Radial (0), Bilateral (1).
- 43. Shape-** Sub-oblate (0), Oblate (1), Oblate-spheroidal (2), Spheroidal (3), Prolate-spheroidal (4), Sub-prolate (5).
- 44. Number of aperture-** 3 (0), 4 (1), Multi (2).
- 45. Type of aperture-** Porate (0), Colpate (1), Colporate (2).
- 46. Exine ornamentation-** Psilate (0), Echinata (1), Lophate (2), Scabrate (3), Granulate (4).
- 47. Cavea-** Absent (0), Present (1).
- 48. Pollen size class-** Minutae (0), Mediae (1), Magnae (2).
- 49. Size of Pollen-** <23.1(0), 23.1-30.1(1), 30.1-37.1 (2), 37.1-44.1 (3), 44.1-51.1 (4), 51.1-58.1 (5), 58.1-65.1 (6), 65.1-72.1 (7), 72.1-79.1 (8), > 79.1 (9).
- 50. Length of Ornamentation-** < 2.4 (0), 2.4-4.8 (1), 4.8-7.2 (2), 7.2-9.6 (3), 9.6-12 (4), 12-14.4 (5), 14.4-16.8 (6), 16.8-19.2 (7), 19.2-21.6 (8), > 21.6 (9).
- 51. Polar Axis (PA)-** < 22.4 (0), 22.4-28.2 (1), 28.2-34 (2), 34-39.8 (3), 39.8-45.6 (4), 45.6-51.4 (5), 51.4-57.2 (6), 57.2-63 (7), 63-68.8 (8), > 68.8 (9).
- 52. Equatorial Diameter (ED)-** < 24.2 (0), 24.2-32.8 (1), 32.8-41.4 (2), 41.4-50 (3), 50-58.6 (4), 58.6-67.2 (5), 67.2-75.8 (6), 75.8-84.4 (7), 84.4-93 (8), > 93 (9).
- 53. PA/ED-** < 0.75 (0), 0.75-0.8 (1), 0.8-0.85 (2), 0.85-0.9 (3), 0.9-0.95 (4), 0.95-1 (5), 1-1.05 (6), 1.05-1.1 (7), 1.1-1.15 (8), > 1.15 (9).
- 54. Size/Width of Aperture-** < 5 (0), 5-7.9 (1), 7.9-10.8 (2), 10.8-13.7 (3), 13.7-16.6 (4), 16.6-19.5 (5), 19.5-22.4 (6), 22.4-25.3 (7), 25.3-28.2 (8), >2 8.2 (9).
- 55. Density of Spines-** < 8.1 (0), 8.1-16.2 (1), 16.2-24.3 (2), 24.3-32.4 (3), 32.4-40.5 (4), 40.5-48.6 (5), 48.6-56.7 (6), 56.7-64.8 (7), 64.8-72.9 (8), > 72.9 (9).

**56. Density of Lacunae-** < 2.3 (0), 2.3-4.6 (1), 4.6-6.9 (2), 6.9-9.2 (3), 9.2-11.5 (4), 11.5-13.8 (5), 13.8-16.1 (6), 16.1-18.4 (7), 18.4-20.7 (8), > 20.7 (9).

Table 3.1 Matrix of Morphological and Palynological Character Codes

Species	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25
<i>Acanthospermum hispidum</i>	3	2	2	1	3	1	3	1	1	1	4	2	0	4	1	4	1	0	1	2	2	0	1	1	1
<i>Acilepis divergens</i>	5	1	1	0	1	1	0	1	5	1	2	1	0	4	3	4	1	0	0	2	0	0	0	0	0
<i>Acmella ciliata</i>	3	1	2	0	3	1	2	3	0	1	4	2	0	4	1	4	5	1	1	2	2	0	1	0	2
<i>Acmella oleracea</i>	3	1	2	0	3	1	2	4	0	1	2	1	0	3	1	4	5	1	0	2	0	0	0	0	0
<i>Acmella paniculata</i>	3	1	1	0	2	0	2	4	0	1	2	1	0	3	1	4	5	1	0	2	0	0	0	0	0
<i>Acmella radicans</i>	3	1	2	0	3	0	2	4	0	1	2	1	0	3	1	4	5	1	0	2	0	0	0	0	0
<i>Acmella uliginosa</i>	3	1	2	0	3	0	2	4	5	1	4	2	0	3	1	4	5	1	1	2	2	0	1	0	2
<i>Adenostemma viscosum</i>	3	1	1	0	1	1	0	4	5	1	2	1	0	4	2	4	1	0	0	2	0	0	0	0	0
<i>Ageratum conyzoides</i>	3	1	2	0	3	1	0	4	5	1	2	1	0	4	1	4	3	0	0	2	0	0	0	0	0
<i>Baccharoides anthelmintica</i>	3	1	1	0	3	1	0	4	0	1	2	1	1	4	3	3	5	0	0	2	0	0	0	0	0
<i>Bidens bipinnata</i>	3	0	2	1	3	1	0	4	0	1	4	3	0	4	2	4	1	0	1	2	2	0	2	0	2
<i>Bidens biternata</i>	3	1	2	1	1	1	0	1	0	1	4	3	0	4	2	4	1	0	1	2	2	0	1	0	2
<i>Blainvillea acmella</i>	3	1	2	0	3	1	0	4	0	1	4	2	0	4	2	4	1	1	1	2	2	0	1	0	1
<i>Blumea axillaris</i>	3	1	1	0	4	1	2	4	4	1	3	2	0	4	3	1	3	0	2	2	1	0	1	0	2
<i>Blumea belangeriana</i>	3	1	1	0	5	1	2	1	0	1	3	2	0	4	3	1	3	0	2	2	1	0	1	0	2
<i>Blumea bovei</i>	3	1	1	0	5	1	2	5	5	1	3	2	0	4	3	1	3	0	2	2	1	0	1	0	2
<i>Blumea eriantha</i>	3	1	1	0	4	1	0	4	4	1	3	2	0	4	3	1	3	0	2	2	1	0	1	0	2
<i>Blumea malcolmii</i>	3	1	1	0	1	1	2	4	2	1	3	2	0	4	3	1	3	0	2	2	1	0	1	0	2

Species	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25
<i>Blumea membranacea</i>	3	1	1	0	6	1	0	1	2	1	3	2	0	4	3	1	3	0	2	2	1	0	1	0	2
<i>Blumea obliqua</i>	3	1	2	0	6	3	2	1	4	1	3	2	0	4	3	1	3	0	2	2	1	0	1	0	2
<i>Blumea oxyodonta</i>	2	1	1	0	4	1	0	4	6	1	3	2	0	4	3	1	3	0	2	2	1	0	1	0	2
<i>Blumea sinuata</i>	3	1	1	0	6	6	0	1	2	1	3	2	0	4	3	1	3	0	2	2	1	0	1	0	2
<i>Blumea virens</i>	3	1	1	0	1	1	0	4	2	1	3	2	0	4	3	1	3	0	2	2	1	0	1	0	2
<i>Caesulia axillaris</i>	3	1	1	0	0	1	0	2	0	1	2	1	1	4	1	4	1	0	0	2	0	0	0	0	0
<i>Centratherum punctatum</i>	1	1	1	0	5	1	0	1	0	1	2	1	1	4	3	4	3	0	0	2	0	0	0	0	0
<i>Chromolaena odorata</i>	5	1	2	0	3	1	1	4	5	1	2	1	0	4	3	1	1	0	0	2	0	0	0	0	0
<i>Cosmos caudatus</i>	3	1	2	1	3	0	1	1	5	1	4	3	0	4	2	4	5	1	1	2	2	0	2	0	2
<i>Cosmos sulphureus</i>	3	1	2	0	6	0	0	1	0	1	4	3	0	4	2	4	5	1	1	2	2	0	2	0	2
<i>Crassocephalum crepidioides</i>	3	1	1	0	6	1	0	1	5	1	2	1	1	4	1	1	1	0	0	2	0	0	0	0	0
<i>Cyanthillium cinereum</i>	3	1	1	0	5	1	2	1	5	1	2	1	0	4	3	4	3	0	0	2	0	0	0	0	0
<i>Cyathocline purpurea</i>	3	1	1	0	6	1	0	5	5	1	3	2	0	2	1	4	1	0	2	2	1	0	1	0	2
<i>Echinops echinatus</i>	5	1	1	0	6	5	3	0	0	2	2	1	0	4	3	5	7	0	0	2	0	0	0	0	0
<i>Eclipta prostrata</i>	1	1	2	0	1	0	2	4	0	1	4	2	0	4	2	4	1	1	1	2	2	0	1	0	2
<i>Elephantopus scaber</i>	0	0	0	0	5	3	2	1	5	2	2	1	1	1	2	1	1	0	0	2	0	0	0	0	0
<i>Emilia sonchifolia</i>	1	1	1	0	6	2	2	0	5	1	2	1	0	4	1	1	3	0	0	2	0	0	0	0	0
<i>Erigeron bonariensis</i>	3	1	1	0	0	0	2	1	2	1	3	2	0	4	3	1	3	0	2	2	1	0	1	0	2
<i>Eschenbachia stricta</i>	3	1	1	0	0	1	2	2	5	1	3	2	0	4	3	4	3	0	2	2	1	0	1	0	2
<i>Gamochaeta pensylvanica</i>	3	1	1	0	5	0	2	1	4	1	3	2	0	4	3	4	1	0	2	2	1	0	1	0	2

Species	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25
<i>Gamochoaeta purpurea</i>	1	1	1	0	5	0	2	1	3	1	3	2	0	4	3	4	3	0	2	2	1	0	1	0	2
<i>Glossocardia bosvallia</i>	2	2	1	0	0	0	2	1	0	1	4	2	0	4	2	2	7	0	1	2	2	0	1	0	2
<i>Goniocaulon indicum</i>	3	1	1	0	0	1	0	2	5	1	2	1	0	4	3	4	3	0	0	2	0	0	0	0	0
<i>Grangea maderaspatana</i>	2	0	1	0	6	1	2	5	0	1	2	1	0	4	1	4	5	0	0	2	0	0	0	0	0
<i>Guizotia abyssinica</i>	3	1	2	0	1	0	0	2	0	1	4	2	0	4	2	4	5	1	1	2	2	0	1	0	2
<i>Helichrysum indicum</i>	3	1	1	0	5	0	2	1	3	1	3	2	0	4	3	5	1	0	2	2	1	0	1	0	2
<i>Lagascea mollis</i>	3	1	2	0	3	5	0	4	0	1	2	1	0	4	2	4	1	0	0	2	0	0	0	0	0
<i>Launaea procumbens</i>	1	1	1	0	6	2	0	1	1	1	1	1	0	4	3	1	1	0	0	1	0	1	0	0	0
<i>Launaea sarmentosa</i>	0	0	0	0	6	2	2	1	4	1	1	1	0	4	3	1	1	0	0	1	0	1	0	0	0
<i>Lipoblepharis urticifolia</i>	6	1	2	0	3	1	1	4	0	1	4	2	0	4	2	4	1	1	1	2	2	0	1	0	2
<i>Lipochaeta lobata</i>	4	1	2	0	2	1	1	6	5	1	4	2	0	4	3	4	1	1	1	2	2	0	1	0	2
<i>Oligochaeta divaricata</i>	3	2	1	0	0	4	3	2	0	1	2	1	0	4	3	4	6	0	0	2	0	0	0	0	0
<i>Parthenium hysterophorus</i>	3	1	1	0	6	0	2	1	5	1	4	2	0	4	1	2	5	0	1	2	2	0	1	1	1
<i>Phyllocephalum microcephalum</i>	3	1	1	0	1	1	0	4	5	1	2	1	1	4	3	2	1	0	0	2	0	0	0	0	0
<i>Pluchea ovalis</i>	5	1	1	0	4	1	0	5	5	1	3	2	0	4	3	4	3	0	2	2	1	0	1	0	2
<i>Pluchea wallichiana</i>	5	1	1	0	2	1	2	5	5	1	3	2	0	4	3	4	1	0	2	2	1	0	1	0	2
<i>Pseudoconyza viscosa</i>	3	1	1	0	1	1	0	5	2	1	3	2	0	4	3	1	3	0	2	2	1	0	1	0	2
<i>Pulicaria angustifolia</i>	1	1	1	0	5	1	0	1	0	1	4	2	0	4	3	5	1	0	1	2	2	0	1	0	2
<i>Sclerocarpus africanus</i>	3	1	2	0	3	1	0	4	0	1	4	3	0	4	1	1	5	0	1	2	2	0	2	0	1
<i>Senecio bombayensis</i>	3	1	1	0	3	1	0	4	5	1	4	3	1	4	1	5	2	0	1	2	2	0	2	0	2

Species	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25
<i>Sonchus asper</i>	3	1	1	0	6	4	3	0	0	1	1	1	0	4	3	1	1	0	0	1	0	1	0	0	0
<i>Sonchus oleraceus</i>	3	1	1	0	3	2	0	0	6	1	1	1	0	4	3	1	1	0	0	1	0	1	0	0	0
<i>Sphaeranthus indicus</i>	2	2	1	0	5	1	2	5	0	2	3	2	0	3	1	4	1	0	2	2	1	0	1	0	3
<i>Sphagneticola trilobata</i>	2	1	2	0	7	1	0	1	0	1	4	2	0	4	2	4	1	1	1	2	2	0	1	0	2
<i>Synedrella nodiflora</i>	3	2	2	0	3	1	0	4	0	1	4	2	0	4	2	4	5	0	1	2	2	0	1	0	2
<i>Thespis thakeri</i>	5	1	1	0	1	1	0	1	5	1	3	2	0	4	1	4	2	0	2	2	1	0	1	1	0
<i>Tithonia diversifolia</i>	5	1	1	0	7	6	0	7	6	1	4	3	0	4	3	4	4	1	1	2	2	0	2	0	2
<i>Tricholepis glaberrima</i>	3	1	1	0	0	1	2	2	0	1	2	1	0	4	3	4	6	0	0	2	0	0	0	0	0
<i>Tridax procumbens</i>	1	1	2	0	3	1	0	6	0	1	4	2	0	4	2	4	5	1	1	2	2	0	1	0	2
<i>Vicoa cernua</i>	3	1	1	0	2	1	0	2	2	1	4	2	0	4	3	1	2	0	3	2	2	0	1	0	2
<i>Vicoa indica</i>	3	1	1	0	0	3	0	2	5	1	4	2	0	4	3	1	1	0	3	2	2	0	1	0	2
<i>Xanthium strumarium</i>	3	1	1	0	7	6	2	7	3	1	2	4	0	5	1	4	1	1	0	2	0	0	0	0	0
<i>Scaevola taccada</i>	5	1	1	0	5	0	2	1	4	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0

Species	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47
<i>Acanthospermum hispidum</i>	2	1	1	0	1	0	2	0	0	4	4	0	2	1	1	0	0	2	0	2	1	0
<i>Acilepis divergens</i>	2	0	0	1	0	0	1	0	0	3	0	1	1	1	1	0	0	2	0	0	2	0
<i>Acmella ciliata</i>	2	0	0	1	3	0	0	0	1	3	2	0	0	0	0	0	0	4	0	1	1	0
<i>Acmella oleracea</i>	2	0	0	0	3	0	2	0	1	3	2	0	0	0	0	0	0	3	0	1	1	0
<i>Acmella paniculata</i>	2	0	0	0	3	0	2	0	1	3	2	0	2	1	1	0	0	4	0	1	1	0
<i>Acmella radicans</i>	1	0	0	0	3	0	4	0	1	3	5	0	0	0	0	0	0	2	0	2	1	0
<i>Acmella uliginosa</i>	1	0	0	1	3	0	2	0	1	3	2	0	0	0	0	0	0	2	0	1	1	1
<i>Adenostemma viscosum</i>	2	0	0	0	3	0	2	0	0	3	3	0	6	1	1	0	0	2	0	2	1	1
<i>Ageratum conyzoides</i>	2	0	0	0	0	0	2	0	0	3	0	1	5	1	1	0	0	2	0	2	1	1
<i>Baccharoides anthelmintica</i>	2	0	0	1	0	0	2	0	0	3	2	1	5	2	2	0	0	4	0	0	1	0
<i>Bidens bipinnata</i>	2	0	0	0	0	0	0	0	0	1	0	1	3	1	1	0	0	4	0	2	1	1
<i>Bidens biternata</i>	2	0	0	0	3	0	0	0	0	1	0	1	3	1	1	0	0	2	0	2	1	0
<i>Blainvillea acmella</i>	2	0	0	0	1	0	0	0	1	3	2	0	2	1	1	0	0	2	0	2	1	1
<i>Blumea axillaris</i>	2	1	0	2	3	1	2	0	0	1	2	1	1	1	1	0	0	2	0	2	1	0
<i>Blumea belangeriana</i>	2	1	0	2	3	1	2	0	0	1	2	1	1	1	1	0	0	2	0	2	1	0
<i>Blumea bovei</i>	2	1	0	1	3	1	2	0	0	1	0	1	1	1	1	0	0	2	0	2	1	1
<i>Blumea eriantha</i>	2	1	0	2	3	1	2	0	0	1	2	1	1	1	1	0	0	1	0	2	1	0
<i>Blumea malcolmii</i>	2	1	0	2	3	1	2	0	0	3	2	1	1	1	1	0	0	4	0	1	1	0
<i>Blumea membranacea</i>	2	1	0	2	3	1	2	0	0	1	2	1	1	1	1	0	0	2	0	2	1	0
<i>Blumea obliqua</i>	2	1	0	2	3	1	2	0	0	1	0	1	1	1	1	0	0	2	0	2	1	0

Species	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47
<i>Blumea oxyodonta</i>	2	1	0	2	3	1	2	0	0	1	0	1	1	1	1	0	0	2	0	2	1	0
<i>Blumea sinuata</i>	2	1	0	2	3	1	2	0	0	1	2	1	1	1	1	0	0	2	0	2	1	0
<i>Blumea virens</i>	2	1	0	2	3	0	2	0	0	3	2	1	1	1	1	0	1	2	0	2	1	0
<i>Caesulia axillaris</i>	2	0	0	2	3	0	4	0	0	3	2	0	5	1	1	0	0	2	0	2	1	0
<i>Centratherum punctatum</i>	2	0	0	1	0	0	0	0	0	3	0	0	5	1	1	0	0	2	0	1	2	0
<i>Chromolaena odorata</i>	2	0	0	0	3	0	2	0	0	3	2	1	1	1	1	0	0	4	0	2	1	1
<i>Cosmos caudatus</i>	2	0	0	0	0	0	0	0	0	4	3	1	3	1	1	0	0	2	0	2	1	1
<i>Cosmos sulphureus</i>	2	0	0	0	0	0	0	0	0	3	0	1	3	1	1	0	0	2	0	2	1	0
<i>Crassocephalum crepidioides</i>	2	0	0	0	0	0	0	0	0	0	0	0	1	1	1	0	0	4	0	2	1	1
<i>Cyanthillium cinereum</i>	2	0	0	1	3	0	0	0	0	3	2	1	1	2	2	0	0	2	0	2	2	0
<i>Cyathocline purpurea</i>	2	0	0	0	3	1	2	0	0	3	0	0	0	0	0	0	0	4	0	2	1	0
<i>Echinops echinatus</i>	2	0	0	1	0	0	2	0	0	3	0	0	4	1	1	0	1	1	0	1	3	0
<i>Eclipta prostrata</i>	2	0	0	1	3	1	2	0	0	3	0	0	0	0	0	0	0	2	0	2	1	1
<i>Elephantopus scaber</i>	2	0	0	1	1	0	2	0	0	3	1	0	1	1	1	0	0	2	0	0	2	0
<i>Emilia sonchifolia</i>	2	0	0	0	3	0	3	0	0	1	3	0	1	1	1	0	0	3	0	2	1	1
<i>Erigeron bonariensis</i>	2	0	0	0	0	1	3	0	0	3	0	0	1	1	1	0	0	0	0	2	1	1
<i>Eschenbachia stricta</i>	2	0	0	0	0	0	3	0	0	3	2	1	1	1	1	0	0	4	0	1	1	0
<i>Gamochaeta pensylvanica</i>	2	0	0	2	3	0	4	0	0	1	0	0	1	1	1	0	0	2	0	2	1	1
<i>Gamochaeta purpurea</i>	2	0	0	2	3	0	4	0	0	1	2	0	1	1	1	0	0	2	0	2	1	1
<i>Glossocardia bosvallia</i>	2	0	0	0	0	1	0	0	0	3	2	1	2	1	1	0	0	2	0	1	1	1

Species	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47
<i>Goniocaulon indicum</i>	2	0	0	1	0	0	2	0	0	3	0	1	5	1	1	0	0	2	0	2	1	1
<i>Grangea maderaspatana</i>	2	1	0	0	1	0	3	0	0	3	3	0	5	1	1	0	0	4	0	2	1	1
<i>Guizotia abyssinica</i>	2	0	0	0	0	1	1	0	0	3	0	1	4	1	1	0	0	2	0	2	1	0
<i>Helichrysum indicum</i>	2	0	0	2	3	1	4	0	0	0	2	0	1	1	1	0	0	2	0	2	1	0
<i>Lagascea mollis</i>	2	0	0	1	3	0	0	0	0	3	2	0	8	1	1	0	0	2	0	1	1	0
<i>Launaea procumbens</i>	2	0	0	2	3	0	2	0	0	0	0	0	1	1	1	0	0	0	0	0	2	0
<i>Launaea sarmentosa</i>	2	0	0	1	3	0	2	0	0	0	0	0	1	1	1	0	0	3	0	0	2	0
<i>Lipoblepharis urticifolia</i>	2	0	0	0	3	0	0	0	1	3	0	1	4	1	1	0	0	2	0	1	1	1
<i>Lipochaeta lobata</i>	1	0	0	0	3	0	0	0	1	3	0	1	4	1	1	0	0	2	0	2	1	1
<i>Oligochaeta divaricata</i>	2	0	0	1	0	0	0	0	0	3	0	0	1	2	1	0	1	5	0	2	0	0
<i>Parthenium hysterophorus</i>	2	0	0	0	0	0	2	0	0	3	0	0	0	0	0	0	0	2	0	1	1	0
<i>Phyllocephalum microcephalum</i>	2	0	0	0	2	0	2	0	0	3	0	0	1	1	1	0	0	2	0	0	2	0
<i>Pluchea ovalis</i>	2	1	0	2	3	1	2	0	0	3	0	1	1	1	1	0	0	2	0	2	1	0
<i>Pluchea wallichiana</i>	2	1	0	2	3	0	2	0	0	3	0	1	1	1	1	0	0	2	0	2	1	0
<i>Pseudoconyza viscosa</i>	2	0	0	1	3	1	2	0	0	3	0	0	1	1	1	0	0	2	0	2	1	0
<i>Pulicaria angustifolia</i>	2	0	0	2	3	0	2	0	0	3	0	1	1	1	1	0	0	4	0	2	1	0
<i>Sclerocarpus africanus</i>	2	0	0	0	2	0	0	0	0	2	1	1	0	0	0	0	0	2	0	2	1	0
<i>Senecio bombayensis</i>	2	0	0	0	3	0	4	0	0	3	3	1	5	1	2	0	0	2	0	2	1	1
<i>Sonchus asper</i>	2	0	0	2	3	0	2	0	0	3	0	1	1	1	1	0	0	4	0	0	2	0
<i>Sonchus oleraceus</i>	2	0	0	1	3	0	2	0	0	3	0	1	1	1	1	0	0	2	0	0	2	0

Species	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47
<i>Sphaeranthus indicus</i>	2	0	0	1	3	0	2	0	1	3	2	0	0	0	0	0	0	2	0	2	1	0
<i>Sphagneticola trilobata</i>	2	0	0	0	3	1	0	0	1	3	0	0	5	1	1	0	0	2	0	2	1	1
<i>Synedrella nodiflora</i>	1	0	0	0	3	0	0	0	1	3	0	1	2	1	1	0	0	2	0	1	1	1
<i>Thespis thakeri</i>	2	0	0	0	3	1	2	0	0	3	0	0	1	1	1	0	0	4	0	2	1	1
<i>Tithonia diversifolia</i>	2	0	0	0	3	0	0	0	0	3	2	0	5	1	1	0	0	2	0	1	1	0
<i>Tricholepis glaberrima</i>	2	0	0	1	0	0	2	0	0	3	0	0	5	1	1	0	0	2	0	2	1	0
<i>Tridax procumbens</i>	2	0	0	1	0	0	0	0	0	3	2	1	8	1	1	0	0	2	0	2	1	0
<i>Vicoa cernua</i>	2	0	0	2	3	0	2	0	0	3	2	1	1	1	1	0	0	4	0	2	1	0
<i>Vicoa indica</i>	2	0	0	2	3	0	2	0	0	3	2	1	1	1	1	0	0	2	0	2	1	0
<i>Xanthium strumarium</i>	0	1	1	0	4	0	0	1	0	4	0	0	0	0	0	0	0	4	0	0	3	0
<i>Scaevola taccada</i>	2	0	0	0	3	0	4	2	0	5	0	0	7	1	1	0	0	3	0	2	4	0

Species	48	49	50	51	52	53	54	55	56
<i>Acanthospermum hispidum</i>	0	0	1	0	0	4	0	3	0
<i>Acilepis divergens</i>	1	2	3	3	2	5	1	9	5
<i>Acmella ciliata</i>	0	0	1	1	0	6	0	4	0
<i>Acmella oleracea</i>	0	1	1	1	0	6	0	4	0
<i>Acmella paniculata</i>	1	1	2	2	1	8	0	3	0
<i>Acmella radicans</i>	1	2	2	2	1	5	1	7	0
<i>Acmella uliginosa</i>	1	1	2	1	1	5	0	4	0
<i>Adenostemma viscosum</i>	1	1	1	1	1	4	1	4	0
<i>Ageratum conyzoides</i>	0	0	1	0	0	5	0	6	0
<i>Baccharoides anthelmintica</i>	1	4	4	5	3	6	1	9	3
<i>Bidens bipinnata</i>	1	1	2	2	1	6	0	5	0
<i>Bidens biternata</i>	1	1	1	2	1	4	0	4	0
<i>Blainvillea acmella</i>	1	1	2	1	1	4	0	4	0
<i>Blumea axillaris</i>	0	0	1	0	0	5	1	3	0
<i>Blumea belangeriana</i>	0	0	2	0	0	4	0	3	0
<i>Blumea bovei</i>	1	1	2	1	1	4	1	3	0
<i>Blumea eriantha</i>	0	0	2	0	0	0	1	3	0
<i>Blumea malcolmi</i>	1	1	1	2	1	6	1	4	0
<i>Blumea membranacea</i>	1	1	1	1	1	5	1	4	0
<i>Blumea obliqua</i>	0	0	1	0	0	4	0	3	0
<i>Blumea oxyodonta</i>	1	2	2	2	1	5	1	2	0
<i>Blumea sinuata</i>	0	0	1	0	0	4	0	3	0
<i>Blumea virens</i>	0	1	1	1	0	5	1	4	0
<i>Caesulia axillaris</i>	1	1	2	1	1	5	0	3	0
<i>Centratherum punctatum</i>	1	3	3	3	2	5	1	9	9
<i>Chromolaena odorata</i>	1	1	2	2	1	6	0	4	0
<i>Cosmos caudatus</i>	1	2	2	2	2	5	1	5	0
<i>Cosmos sulphureus</i>	1	1	2	1	1	5	0	3	0
<i>Crassocephalum crepidioides</i>	1	1	1	2	1	7	0	6	0
<i>Cyanthillium cinereum</i>	1	2	1	2	1	5	1	6	2
<i>Cyathocline purpurea</i>	0	0	1	0	0	7	0	3	0
<i>Echinops echinatus</i>	2	9	9	9	9	0	9	0	0

Species	48	49	50	51	52	53	54	55	56
<i>Eclipta prostrata</i>	0	0	1	0	0	5	0	3	0
<i>Elephantopus scaber</i>	1	4	2	4	3	5	1	8	3
<i>Emilia sonchifolia</i>	1	1	1	1	1	6	0	4	0
<i>Erigeron bonariensis</i>	0	0	1	0	0	3	0	5	0
<i>Eschenbachia stricta</i>	0	1	1	1	0	7	0	4	0
<i>Gamochaeta pensylvanica</i>	0	0	0	0	0	4	0	5	0
<i>Gamochaeta purpurea</i>	0	0	0	0	0	4	0	5	0
<i>Glossocardia bosvallia</i>	1	1	2	2	1	5	0	3	0
<i>Goniocaulon indicum</i>	1	3	1	3	2	5	2	4	0
<i>Grangea maderaspatana</i>	0	1	1	1	1	6	0	3	0
<i>Guizotia abyssinica</i>	1	1	2	2	1	5	0	5	0
<i>Helichrysum indicum</i>	0	0	0	0	0	3	0	6	0
<i>Lagascea mollis</i>	0	1	1	1	0	5	0	4	0
<i>Launaea procumbens</i>	1	2	2	2	2	1	1	6	2
<i>Launaea sarmentosa</i>	1	1	2	1	1	6	1	6	2
<i>Lipoblepharis urticifolia</i>	1	2	2	2	2	4	0	3	0
<i>Lipochaeta lobata</i>	1	1	3	1	1	3	0	5	0
<i>Oligochaeta divaricata</i>	1	1	0	2	1	9	0	0	0
<i>Parthenium hysterophorus</i>	0	0	0	0	0	5	0	2	0
<i>Phyllocephalum microcephalum</i>	2	6	2	7	5	5	1	6	2
<i>Pluchea ovalis</i>	0	0	2	1	0	5	1	3	0
<i>Pluchea wallichiana</i>	1	1	1	1	1	5	1	4	0
<i>Pseudoconyza viscosa</i>	0	0	1	0	1	4	1	4	0
<i>Pulicaria angustifolia</i>	0	1	1	1	0	6	0	3	0
<i>Sclerocarpus africanus</i>	1	1	2	2	1	5	0	7	0
<i>Senecio bombayensis</i>	1	1	1	1	1	4	0	4	0
<i>Sonchus asper</i>	0	1	1	1	0	6	1	6	2
<i>Sonchus oleraceus</i>	1	2	2	2	2	5	0	6	3
<i>Sphaeranthus indicus</i>	0	0	2	1	0	5	0	3	0
<i>Sphagneticola trilobata</i>	0	1	1	1	1	4	0	4	0
<i>Synedrella nodiflora</i>	1	1	1	1	1	5	0	5	0
<i>Thespis thakeri</i>	0	0	0	0	0	8	0	4	0

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Species	48	49	50	51	52	53	54	55	56
<i>Tithonia diversifolia</i>	1	2	3	3	2	5	1	5	0
<i>Tricholepis glaberrima</i>	1	3	2	3	2	5	2	4	0
<i>Tridax procumbens</i>	1	1	1	1	1	5	1	5	0
<i>Vicoa cernua</i>	0	0	2	0	0	6	1	4	0
<i>Vicoa indica</i>	0	0	1	0	0	5	0	3	0
<i>Xanthium strumarium</i>	0	0	0	1	0	7	0	8	0
<i>Scaevola taccada</i>	2	8	0	9	6	6	2	0	0

### 3.4.2 Preparation of Cladogram

Nexus file of above characters were prepared. This nexus file was run in PAUP\* software (Swofford, 2003). Maximum parsimony analyses were conducted for phylogenetic analysis. Heuristic search in parsimony method was used with 100 replicates. Minimum 50% major-rule consensus tree was created and save. Bootstrap support (BS) was estimated using 100 replicates. This tree was then compared along with supertree of Asteraceae family prepared by Funk *et al.* (2005).