DISCUSSION

DISCUSSION

The prominence of flavones and reduction in flavonols and proanthocyanidins make the family Acanthaceae one of the advanced groups in the Scrophulariales. The pattern of distribution of flavonoids and phenolic acids do suggest the recognition of 3 distinct taxonomic groups.

The Nelsonioideae are characterised by the uniform presence of 6-deoxyflavones, aucubins, proanthocyanidins; rare occurence of p-OH benzoic acid and absence of 6-oxygenated flavones and flavonols. The characteristic 6-deoxyflavones of the Nelsonioideae are apigenin, luteolin and 3'-OMe luteolin; benzoic acids, such as, vanillic, syringic and melilotic acids and cinnamic acids like caffeic and sinapic acids. The subfamily Nelsonioideae is similar to the Thunbergioideae in the absence of flavonols, 6-oxyflavones and p-OH benzoic acids and in other characters like presence of panduaraeform glandular hairs and absence of cystoliths of the Acanthoideae. This group differs from the Thunbergioideae in having aucubins, proanthocyanidins and cinnamic acids, all of which are seen in Acanthoideae also. The absence of 6-oxyflavones which are the characteristic compounds of the Rhinantheae does not support the placement of Nelsonioideae in the Scrophulariaceae. Similar conclusions are drawn by other workers based on characters such as cuticular observations (Ahmad, 1974), foliar anatomy (Nafday, 1965), pollen-grains (Bhaduri, 1944), assymetrical growth of endosperm (Mohan Ram and Masand, 1963) and non-functional jaculators. The Nelsonioideae are clearly a homogenous group of the Acanthaceae having equal affinities with the Thunbergioideae and Acanthoideae. The absence of 6-oxyflavones keeps this group primitive over Acanthoideae, but it occupies a higher position than the Thunbergioideae in eliminating glycoflavones. Though the ditinctness of the Nelsonioideae is emphasised by almost all the workers, it is surprising that none of the authors proposed a family status for this group. They possess the same identity as that of the Thunbergiaceae and therefore these exists no reasons why this group should not be treated as a family, the Nelsoniaceae.

The evidence from the present work suggest that both *Nelsonia canescence* (Lamk.) Spreng and *N. campestris* Br. are very similar in chemical constitution and these names should be treated as synonyms.

The Thunbergioideae are chemically distinct by the presence of glycoflavones and non-occurence of 6-oxygenated flavones, proanthocyanidins, aucubins and cinnamic acids. The presence of protocatechuic, syringic and gentisic acids with vitexin and isovitexin forms the unifying characters of the group. The group does not possess the characteristic compounds of the Acanthaceae i.e. the 6-oxyflavones, aucubins and proanthocynidins. Absence of 6-oxyflavones in Thunbergioideae is a clear evidence of the difference with the Rhinantheae of Scrophulariaceae with which it is sometimes grouped. The seperate identity of the Thunbergioideae is also evident in other characters such as the climbing habit, prominent bracteoles, axillary flowers, a small-sized 10-14 toothed calyx, panduarae form glandular hairs (Ahmad, 1974) and cushion-shaped funiculus forming a sort of obturator. A conspectus of all these characters justify the recognition of this subfamily as a separate family, the Thunbergiaceae. The Thunbergiaceae are similar to the Acanthaceae in features like zygomorphic androecium, exalbuminous seeds, conspicuous and cushion or annular disc etc. and therefore finds the later family as the closest in the Scrophulariales. This family is primitive to the Acanthaceae in elaborating glycoflavones and in the absence of advanced 6-oxyflavones and aucubins. Its relationship with the Nelsonioideae is already explained above.

The third group, subfamily Acanthoideae, is relatively homogenous, in synthesising 6-deoxyflavones, 6-oxyflavones, proanthocyanidins and aucubins and reducing the emphasis on flavonols and glycoflavones. Phenolic acids such as p-OH benzoic, vanillic, syringic, ferulic and p-coumaric acids are the other characters of this subfamily.

The two groups proposed by Lindau (1895) within the Acanthoideae **i.e., Contortae** and **Imbricatae**, also get some support from the chemical evidence. The **Contortae** comprising of the tribe Trichanthereae, Louteridieae, Hygrophileae, Petalidieae, Strobilantheae, Ruellieae and Barlerieae do have a much higher concentration of 6-oxyflavones (44%) with almost near elimination of glycoflavones (2%) and complete loss of flavonols. As against this, the **Imbricatae** consisting of Acantheae, Aphelandreae, Andrographideae, Asystasieae, Graptophylleae, Pseuderanthemeae, Odontonemeae, Isoglossieae and Justicieae possess very little 6-oxyflavones (8%) and higher incidence of glycoflavones (17%) and flavonols (13%). Whether these groups should be given a status of subfamilies, is to be examined in the light of evidences from other disciplines.

The division of the Acanthaceae *sensu* Bremekamp (which contain only the Acanthoideae of Lindau) to two subfamilies, ***Acanthoideae*** Bremek. and ***Ruellioideae*** Bremek. do not get any support from chemistry. The Acanthoideae, containing the tribes Aphelandreae and Acantheae of the present investigation, are not in any way different from the tribes of Ruellioideae. The treatment of Barlerieae, Strobilantheae, Hygrophileae as subtribes of Ruellieae and Odontoneminae as a subtribe of Justicieae also does not gain any evidence from the distribution of chemical characters. Barlerieae, Strobilantheae and Hygrophileae are chemically separate from each other and from Ruellieae and therfore they should be accorded a tribal status. It is true that all these four tribes possess a number of characters in common, but this evidences their co-evolution. Incidentally, these tribes form the core group of the Contortae of Lindau. The tribe Lepidagatheae of Bremekamp (l.c.) which is a splinter group of the Barlerieae of Lindau, is very similar to the rest of the Barlerieae and therfore do not possess a chemical identity. This invalidates the tribal status accorded to the Lepidagatheae which has to be merged with Barlerieae.

Within the Contortae, all the tribes possess similar assortment of chemical characters. All of them are at the same level of evolution in possessing 6-oxy & 6-deoxy flavones and eliminating flavonols, glycoflavones and proanthocyanidins.

The tribe Hygrophileae with its five plants screened here appear to be very homogenous. *Hygrophila spinosa* which was transferred to a new genus *Asteracantha* (*A. longifolia*) is exactly similar to the other species of *Hygrophila* in containing 6-oxyflavones alongwith 6-deoxyflavones. This cast serious doubts on the distinct identity of this plant as a separate genus and therefore based on the chemistry its retention in the genus *Hygrophila* as *H. spinosa* is advocated.

The Strobilantheae are more or less similar to the Hygrophileae in their chemistry. This tribe is uniform in containing 4'-OMe scutellarein (6-oxyflavone) and characteristic phenolic acids.

The chemical difference between *Hemigraphis* and *Ruellia* can be compared with *R. tuberosa* (the species on which *Ruellia* is founded by Linneus) which consist of 7-OMe apigenin and scutellarein towards advancement. *Hemigraphis latebrosa* Var. *heyneana* Bremek. and *H.elegans* Var. *crenata* Clarke are separated by 6-OCH_3 scutellarein and syringic acid to make the later advanced over the former whereas *H.hirta* T.Anders. has found to be primitive to both.

Gantelbua, a monotypic genus separated from *Hemigraphis* possess 7-OMe apigenin as the additional character. This suppliments its peculiar structure of inflorescence, deeply divided calyx and small size of areola to make the species unique and therefore the generic status given to *Gantelbua* is supported on chemical grounds.

Transfer of *Goldfusia dalhousiana* Nees to *Strobilanthes*

Dalhousianus Clarke. seems to be in order because this plant shows presence of 4'-OMe apigenin and 6-OMe scutellarein, the typical chemical of Strobilanthes.

Strobilanthes, as Cramer (1992) indicated, is a difficult genus. Cytopalynological evidences (Valsaladevi, 1987) also suggest that the genus is highly heterogenus. Bremekamp (1994) have splitted the genus in to about two dozen genera. According to Ahmad (1974d) the epidermal characters or most of the taxa under this tribe do not indicate striking dissimilarities which would help to separate them under the new genera of Bremekamp (l.c.) whereas Vishnu Mittre and Gupta (1966) said, "it is indeed difficult to commend on the delimitation by Bremekamp and to remark how far he has succeeded through the segregation of *Strobilanthes* Bl. in to several genera in raising it from an artificial status to a natural one."

The present study reveals groups of plants. This in correlation with Bremekamp (l.c.). With regards to 4'-OMe apigenin and 6-OMe scutellarein, it is interesting to note that the *Nilgirianthus* and *Strobilanthes* can be distinguished from each other. The *Carvia callosa* is also found to be eminent in the group possessing scutellarein.

The Ruellieae is found similar to Strobilantheae in not producing flavonols, glycoflavones, proanthocyanidins and aucubins. *Ruellia alba* which is separated from *R. tuberosa* based on the single character i.e. the color of petals by Joshi (1981) does not show any significant difference (not even in the petal-chemistry) from the later plant. *Ruellia patula* and

R. prostrata are found to be distinct from *R.tuberosa* in their chemistry favouring their placement in the genus *Dipteracanthus* as followed Bremekamp (1948), Santapau (1951), Mathew (1983), Barker (1986) and Cramer (1992).

Bentham and Hooker (l.c.) had treated species of *Eranthemum* Linn. in Petalidieae, as *Daedalacanthus*. The presence of apigenin, 4'-OMe apigenin, luteolin justifies the placement of *Eranthemum* Linn. in Ruellieae. The evidences from various other disciplines such as micromorphological characters (Ahmad, 1974,b); palynology (Sharma and Vishnu Mittre, 1983) and cytology (Valsaladevi, 1987) also support this contention.

In containing apigenin and its methoxy derivatives, the tribe Barlerieae is similar to the Ruellieae but differ in possessing the advanced scutellarein and its derivatives. Within the Contorate, the Barleriae and Strobilantheae are the most advanced tribes.

Bentham and Hooker (1876) placed *Barleria* in Justicieae. The presence of a variety of 6-deoxyflavones keep this genus primitive to the other genera of Justicieae containing the 6-oxyflavones. Valsaladevi (1987) remarks that cytologically, various species of *Barleria* Linn., which possess large sized chromosomes are distisnct from other genera of Benthtam and Hooker's Justicieae and hence this separation is reasonable. Based on palynology (Chaubal, l.c.), floral anatomy, cytology and wood anatomy (Datta and Maiti, 1969, 1970, 1971) also favoured this treatment. There is considerable disagreement with regard to the placement of

Lepidagathis and *Barleria* appear to be quite distinct both cytologically and morphologically (Valsaladevi, 1987). According to Ahmad (1975), *Lepidagathis* lacks an important character of *Barleria*, **i.e.** prominant double cystolith which also is reported by Inamdar **et.al.** (1990). Balkwill and Norris (1988) comments that in the corolla-shape, rugula and colporate pollengrains, *Lepidagathis* Willd. is similar to *Hygrophila* Br.

Within the Imbricatae, a number of the tribes are distinct, is possessing different assortment of characters for eg. Tribes Acantheae, Odontonemeae and Justiceae possess both flavonols and glycoflavones while Pseuderanthemae, Graptophylleae, Asystasieae and Aphelandreae are free of both these types of compounds. Andrographideae contain only glycoflavones.

Tribe Acantheae can be separated from Barlerieae by the presence of flavonols, glycoflavones and cinnamic acids and absence of 6-oxyflavones. The Aphelandreae are close to this tribe because of the presence of cinnamic acids along with the other flavonoid components. *Crossandra* was treated variously by different workers with regard to its placement. Seed-morphological features (Gutterman, 1973; Gutterman **et.al**, 1967,1969,1973) suggest this genus to be close to *Blepharis*. It differs from *Acanthus* in stelar structure and petiolar anatomy (De, 1967). A separate subtribe within Acanthaceae is proposed by Balkwill and Norris (1988). The present chemical analysis supports this suggestion.

The absence of 6-oxyflavones, flavonols and aucubins does not support the advanced status attributed to the Andrographideae by Nees (**l.c.**). Bentham and Hooker had transformed this tribe near to the tribe Justicieae. The resemblence between *Andographis* and *Elytraria* in their seed-morphology (Mauritzon, 1934; Mohan Ram, 1960; Mohan Ram and Wadhi, 1965; Pathak and Ambegaonkar, 1955; Johri and Singh, 1959) made Hansen (1985) suggest an adjacent placement of Andrographideae adjacent to the Nelsonieae of Bentham and Hooker (**l.c.**). However, Bremekamp (1965) had rejected the evidences cited by Mauritzon (**l.c.**). Presence of 4'-OMe apigenin, luteolin, 3'-OMe luteolin, vitexin and isovitexin and proanthocyanidins are the characters of Nelsonioideae different from the Andrographideae. *Indonesiella*, the genus separated from *Andrographis* by Sreemadhavan (1968) is not chemically different from the latter genus. However, based on seed-coat surface pattern (Sivarajan, 1983) and other workers (Mathew, 1983, Valsaldevi, **l.c.**) support the retention of this genus separate. *Bremekampia* Sreem. separated from *Halplanthus* Nees. by Sreemadhavan (**l.c.**) is chemically distinct in possessing apigenin. Therefore this genus may be treated valid.

The Asystasieae are a primitive tribe possessing only 6-deoxyflavones and therefore it occupies the same level as those of tribes Pseuderanthemeae and Graptophylleae.

Tribe Odontonemeae, a heterogenus tribe, is found to contain advanced 6-oxyflavones with 6-deoxyflavones and primitive flavonols and glycoflavones. The subtribe

Diclipterinae, containing 6-deoxyflavones and flavonols, is distinct from Andrographideae in not having flavonols and therefore the intermediate position between Eranthemeae and Andrographideae referred by Nees (l.c.) does not gain support on chemical grounds. *Rungia* has been treated variously by different workers majority of them suggest its placement in the tribe Justicieae. *Rungia* is chemically different from *Justicia* in containing apigenin, 3'-OMe luteolin, 7,4'-diOMe luteolin and 3'-OMe quercetin and therefore should be treated as an independant genus.

The subtribe Odontoneminae is different from the Diclipterinae in having 6-oxyflavones and glycoflavones.

The tribe Justicieae is characterised by 4'-OMe apigenin and 6-OMe scutellarein along with variously distributed Kaempferol, vitexin and isovitexin, 4'-OMe vitexin and 6-OMe vitexin. *Adhatoda* Nees. is a controversial taxon since long as to whether it should be merged with *Justicia* or to be retained as a separate genus (Stearn, 1971; Graham, 1988; Cramer, 1992). Favouring the retention of *Adhatoda* Nees. as a distinct genus, Mathew (1982) opines "We prefer to retain this well-known and widely (medicinally) used shrub *Adhatoda* (the genus name itself derived from the local vernacular) as distinct from the herbaceous *Justicia* species despite the current tendency to refer it to the latter." Along with the presence of distinct alkaloids (vasicine and vasicinone), Kaempferol, quercetin and vitexin and isovitexin; epidermal (Ahmad, 1979), Cytological (Grant, 1955) and palynological (Bhaduri, l.c.) characters provide the diagnostic evidences for *Adhatoda* away from *Justicia*. The latter genus is the largest and most complex genus of the family having approximately 420-600 species and needs sub-division (Daniel, 1989) comments on the subdivision of *Justicia* on chemical grounds are reserved till more plants of this genus are surveyed for their constituents.

CLADISTICS :

The cladistic analysis done during the preparation of this thesis and the resultant ambiguity is not new among the taxonomists. In one of the most ambitious cladistic approach Doyle and Donoghue (1987) attempted to find out seed-plant phylogeny and origin of Angiosperms by taking into consideration of 20 taxa. They ended up with 36 most parsimonious cladogram and examined the variations among the trees. They themselves cited several reasons why the classificatory schemes they had proposed should be treated with caution, and concluded that the schemes presented were necessarily speculative and left many questions unanswered. Even at the level of spcies within a genus, cladistics leaves more quesions than answers. For example, Calljas (1986) studied 21 species of *Piper* using 55 characters produced 50 equally parsimonious cladograms each with 76 steps. Any choice that is done among these 50 must be subjective.

Cronquist (1987) in one of the seathing attacks on Cladism reports to have seen a prepublication manuscript calling for 124 steps to achieve several parsimonious cladograms. Within months it was replaced by another manuscript calling for only 123 steps to produce some different most parsimonious cladograms. The number of equally parsimonious trees runs into hundreds (Dahlgren and Bremer,1985), with an even larger number that are only one or two steps longer than the most parsimonious ones. Then the cladist must choose the bone intuitively preferred or accept only those features that are common to most or all of these most parsimonious cladograms leaving the other decisions unresolved. Though Donoghue and Cantino (1988) as a response to the Critisism of Cladism by Cronquist (1987) defended Cladism, their defence was mainly on the concepts of paraphyletic groups. They accepted that cladograms maintain neutrality as to which species extinct or extant are needed to establish such hypothesis (Eldredge and Cracraft, 1980; Wiley, 1981).

In the light of these discussions it is clear that cladistics remains a debatable topic. The study presented in this thesis also does not favour cladistics as a convenient way to achieve a phylogenic classification. However, more studies are to be undertaken before we come to any conclusion on the validity of Cladistics as a viable method of taxonomy.