INTRODUCTION

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The Caryophyllales represent one of the largest orders of Angiosperms. This group, containing 12 families, has been cited as one of the few natural orders of Dicotyledons (Cronquist, 1981). The plants belonging to this order possess unique N-containing pigments, the betalains, and p-type sieve element plastids. Due to their morphological peculiarities such as free central or basal placentation and curved embryo, the Caryophyllales are known as Centrospermae (Eichler, 1876) or Curvembryae (Bentham and Hooker, 1865) respectively.

The order Caryophyllales as visualized by Cronquist (1981) included 10 betalain containing families, Phytolaccaceae, Achatocarpaceae, Nyctaginaceae, Aizoaceae, Didiereaceae, Cactaceae, Chenopodiaceae, Amaranthaceae, Portulacaceae, Basellaceae, and two anthocyanin-containing families the Caryophyllaceae and Molluginaceae. This order forms a major taxon of the subclass Caryophyllidae which also contains two monotypic orders, the Polygonales and Plumbaginales.

The concept of Caryophyllales is not recent. Braun (1864) created "ordung Caryophyllineae" containing eight families, the Nyctaginaceae, Chenopodiaceae, Amaranthaceae, Caryophyllaceae, Phytolaccaceae, Portulacaceae, Aizoaceae and Opuntiaceae. Even now these eight families are considered to be the 'core' families of this order. Of the four additional families incorporated in the present system of classification, the Achatocarpaceae were a segregation of the Phytolaccaceae, the Molluginaceae of the Aizoaceae and the Basellaceae of the Chenopodiaceae. The family Didiereaceae was brought into this grouping by Hallier (1912) along with the Crassulaceae and Polygonaceae. After the discovery of betalains in the family Didiereaceae, Cronquist (1968) and Takhtajan (1959) supported the placement of Didiereaceae in Caryophyllales. The placement of Crassulaceae and Polygonaceae in this order was opposed by Cronquist (1981) and Takhtajan (1980). Bessey (1915) and Thorne (1968) favoured keeping the Polygonaceae in the order Caryophyllales, but they did not agree with placement of Crassulaceae in the Caryophyllales.

The term Caryophyllales, as understood by Bentham and Hooker (1865), is very different from the above concept and contained only two families, the Caryophyllaceae and Portulacaceae. Most of the other families were distributed in the series Curvembryae and one in the series Calyciflorae.

Takhtajan (1980) and Dahlgren (1980) added two more families, the Stegnospermaceae and Hectorellaceae to the

Caryophyllales, increasing the number of families to 14.

Hutchinson (1964) distributed the families of Caryophyllales (Cronquist, 1981) in orders Bixales (Achatocarpaceae), Thymelaeles (Nyctaginaceae), Pittosporales (Stegnospermaceae), Cactales (Cactaceae) in the division Lignosae and Caryophyllales (Elatinaceae, Molluginaceae, Caryophyllaceae, Aizoaceae, Portulacàceae), Polygonales (Polygonaceae, Illicebraceae) and Chenopodiales (Barbeuiaceae, Phytolaccaceae, Gymostemonaceae, Agdestidaceae, Petiveriaceae, Chenopodiaceae, Amaranthaceae, Cynocrambaceae, Batidaceae, Basellaceae) in the division Herbaceae.

Mabry <u>et</u> <u>al</u>.(1963) as well as Behnke and Turner (1971) insisted on grouping the betalain-containing families in suborder Chenopodineae, while keeping the anthocyanin families in another suborder Caryophyllineae. The suborder Chenopodineae included the ten betalain families and suborder Caryophyllineae consisted of the Caryophyllaceae and Molluginaceae. Though Thorne (1968) agrees with this concept, he merges the members of Molluginaceae in the Aizoaceae along with the betalain-producing taxa within the suborder Chenopodineae.

The Cactaceae were first included in this order Caryophyllales by Braun (1864). But Eichler (1978) excluded Cactaceae from Caryophyllales in his final system of classification. Engler (1964) placed the Cactaceae in a separate order Cactales. Soo (1967) also supported this exclusion. But the discovery of betalains in Cactaceae established the Centrospermous affinity of Cactaceae, and a majority of taxonomists (Cronquist, 1981; Takhtajan, 1980; Dahlgren, 1980) strongly favour the idea of keeping Cactaceae in Caryophyllales.

The Theligonaceae, once placed in the order Caryophyllales by Eichler (1878) and Cronquist (1965), are now to be found in the subclass Asteridae under the order Rutales (Cronquist, 1981). This taxa fit well in Rutales than in the Caryophyllales.

The various schemes of classification proposed for the families included in the present work are summarised in Table-22 (presented in Appendix-I)

Members of the order Caryophyllales are herbs or less commonly shrubs or small trees. Leaves alternate, opposite or rarely whorled, simple and commonly entire, some times reduced to spines, often with 'kranz' anatomy. Stipules usually absent, if present very small and reduced.

Flowers in various kinds of infloresence; most commonly entomophilous, perfect but in some cases unisexual. Regular or less commonly irregular, hypogynous to perigynous to epigynous. Sepals (1) 2-5 (-10) distinct or connate below to form a Calyx tube, calyx tube now and then corolloid in appearance. Petals usually absent, if present two to many, distinct or connate sometimes of staminodal origin, occasionally phyletically transformed sepals. Stamens one to many often as many or twice as many as the sepals or petals, when numerous originating in Centrifugal sequence. Anthers mostly tetrasporangiate and dithecal, but bisporangiate and monothecal are not very rare, usually opening by longitudinal slits. Gynoceium of 1- many carpels, these distinct or more often united to form a compound, superior or inferior ovary with distinct styles or with a single, usually lobed or cleft style, the locules as many as the carpels or very often the ovary unilocular, through failure of partitions, placentation variously axile, basal, free-central or parietal. Ovules one to many more or less distinctly campylotropous or amphitropous to seldom hemitropous or even anatropous, crassinucellar, bitegmic or rarely unitegmic. The micropyle commonly formed by the inner integument, endosperm development nuclear. Fruit of various types, dry or fleshy, dehiscent or indehiscent, seeds often arilate, embryo large, nearly always dicotyledonous peripheral, straight, quite often curved or annular, bordering or surrounding the more or less abundant, starchy perisperm, or the perisperm some times scanty or even absent, true endosperm lacking or very scanty.

Embryological features of the Caryophyllales

One of the unique peculiarities of the Caryophyllales is their peculiar embryological features particularly the structure of ovules and seeds (Rochen, 1927; Schnarf, 1933; Mauritzon, 1939). Maheswari critically evaluvated the embryological peculiarities of Caryophyllales and listed them as 1) glandular anther tapetum 2) division of microspore mother cell simultaneous instead of successive (3) trinucleate pollen grains 4) Campylotropous ovules, with strongly curved embryo (amphitropous, seldom anatropous e.g. Pisonia, Aizoaceae) 5) micropyle formed by the swollen apex of the inner integument, which protrude and approches the funiculus 6) a hypodermal archesporial cell which cut off a cell wall 7) formation of a nuclear cap arising from periclinal divisions of the cells of the nucellar epidermis 8) functioning of the chalazal megaspore of the tetrad 9) formation of a monosporic eight-nucleate embryo sac and 10) functioning of the perisperm as the chief storage region. Each of these characters may be found in other orders, and some times several of them occur together. But the occurrence of all these features collectively is not noticed any where else except in the Caryophyllales (Maheswari, 1964; Davis, 1966; De Buhr 1975).

Palynological features of the Caryophyllales

Majority of the Caryophyllales have a characteristic, spherical, pantoporate type of pollen grains, that has been compared to a "golf ball" in appearence. This type of pollen is rare among angiosperms, though not wholly unknown (Nowicke, 1975; Skvarla and Nowicke, 1976). Nowicke (1975) found three common pollen types all with spinulose and tubuliferous/punctate ektexine among the betalain families as also in the Caryophyllaceae and Molluginaceae. Two more related minor types were also detected in the Nyctaginaceae. The typical pantoporate type of pollen is not observed in Achatocarpaceae, Bataceae, Gyrostemonaceae and Polygonaceae.

Cytological features of the Caryophyllales

The basic chromosome number in a majority of phenetically primitive and closely related Caryophyllalean families is x = 9, which form a central stock, from which various lines of evolution occurred and more advanced taxa derived. A trend of dysploid reduction seems to be operative in base number in members of Caryophyllaceae (Illiceaceae, Lychinidae, and Sileneae), Chenopodiaceae and Portulacaceae. In another evolutionary line, particularly in progressive perennial or even woody groups a dysploid (and polyploid) increase of chromosome base number is observed. The trend of chromosome base number increase through disploidy and polyploidy is is common both in betalain producing and anthocyanin producing families (Ehrendorfer, 1976).

Anatomical features of Caryophyllales

Members of this order often are characterized by anomalous secondary growth, producing alternate concetric rings of xylem and phloem.

A unique feature of the Caryophyllales is the presence of P-type sieve element plastids. These plastids contain protein as inclusions as against the normal ones which elaborate starch (S-type). The common type of P-type plastids in Caryophyllales contains peripheral ring-shaped bundles of protein filaments and a central protein crystaloid. The outer ring-shaped protein bundles are consistent in shape and occurrence in almost all the members. The central protein Crystaloid is very variable or even absent in different plant groups within.

Based on the nature of the central protein crystaloid, three different type of P-type plastids are recognised in the Caryophyllales. The most common type of sieve element plastids is with a globular protein crystaloid (Cactaceae, phytolaccaceae, Didiereaceae, Aizoaceae, Molluginaceae, Basellaceae, Portulacaceae, and Nyctaginaceae). Plastids with Polygonal crystaloids are reported from all the investigated members of caryophyllaceae, Stegnosperma (Phytolaccaceae) and Limeum (Molluginaceae). Central protein crystaloids are lacking in plastids of the Amaranthaceae and Chenopodiaceae (Behnke, 1971, 1972, 1976).

After the investigation of sieve element plastids of 14 families, Behnke (1976) proposed a diagram indicative of the phylogenetic relationship existing among members of the Caryophyllales. He placed phytolaccaceae which possess plastids containing proteins as well as starch, and all the families containing different type of p-type plastids are traced back to this family.

Pigments of Caryophyllales

Betalains, a unique type of plant pigments, are restricted to the ten families, the Phytolaccaceae, Achatocarpaceae, Nyctaginaceae, Aizoaceae, Didiereaceae, Cactaceae, Chenopodiaceae, Amaranthaceae, Portulacaceae and Basellaceae belonging to the order Caryophyllales. The Caryophyllaceae and Molluginaceae do not possess the biosynthetic mechanism to produce these compounds but contain anthocyanins which are common flower pigments of the rest of the angiosperms.

Betalains is a collective term for betacyanins and betaxanthins, a class of red, violet and yellow nitrogen-containing pigments. Betacyanins contain both dihydroindole and dihydropyridine rings and a polymethylene chromophore and are red in colour (Wyler et al. 1963), whereas betaxanthins which are yellow in colour contain L-proline and 4-methylpyridine-2,6-dicarboxylic acid (Piattelli *et al.* 1964b). These pigments occur as glycosides and are water soluble. The colour will be destroyed on heating with HCl at 100°C. Betalains exhibit an absorption spectrum in the visible region in the range of $\lambda^{max}_{H_2O}$ - 532-554 nm and 459-471 nm. Their chromatographic mobility is very low and the Rf value in n-butanol : acetic acid : water (4:1:5) is between 0.03-0.06.

Anthocyanins, which are polyphenols (flavonoids), possess the same colour, almost similar absorption maximum ($\lambda^{max}_{MeOH-HCl}$ 520-546 nm) and serve as flower colour pigments. Both these groups of compounds, betalains and anthocyanins are mutually exclusive in the plant Kingdom (Mabry, 1964; Wyler and Dreiding 1957).

Mabry *et al.* (1963) divided the order caryophyllales into suborder Chenopodineae containing the betalain-producing families and suborder Caryophyllineae with anthocyanin-producing families. The presence of betalains was a significant evidence in favour of grouping Cactaceae and Didiereaceae which were otherwise of dubious systematic position. The absence of betalains was the criterion for removal of the Bataceae, Polygonaceae and Theligonaceae from the Caryophyllales.

On the basis of morphological characters, the Phytolaccaceae are considered as the most primitive among the families of the Caryophyllales (Cronquist, 1981; Takhtajan, 1980; Ehrendorfer, 1976; Behnke, 1976.) Data from ultrastructure of sieve element plastids and cytology also support this conclusion and Behnke (1976) derives all other families of this order directly or indirectly from Phytolaccaceae.

But the concept of the apocarpous condition, present in the subgenus Pircunia of Phytolacca and in Giskia, as derived from a primitive Syncarpous pistil and therefore is advanced (Rohweder, 1965, a,b; Hofmann, 1973), is gaining support these days and this casts a serious doubts on the postulated primitiveness of the Phytolaccaceae.

An array of relatively primitive characters such as alternating leaves, sieve element plastids with round protein crystaloids, thyrsoid infloresence, hermophrodite pentamerous flowers with single perianth, two (or three) whorls of stamens and tricolpate pollen grains, five free or partly fused carpels with axillary placentation, numerous ovules and a basic chromosome number X = 9 shared by the Molluginaceae, Aizoaceae and Phytolaccaceae prompted Ehrendorfer (1976) to consider these three families as a group closer to the ancestral stock, from which all other families are derived.

The Amaranthaceae and Chenopodiaceae share a number of characters like Palynological (Pantoporate type of pollen with similarly thickened tecta and aperture structure etc.) (Skvarla and Nowicke, 1976) and sieve element plastids with out protein Crystalloids (Behnke, 1976). These two families are predominantly specialized towards anemophily with progressive reduction of flowers and to the development of an one seeded fruit. The Amaranthaceae are considered slightly advanced over Chenopodiaceae. A direct evolution of the Amaranthaceae from Chenopodiaceae is not favoured by Thakhtajan, who considers the tribe Celosieae of Amaranthaceae is more primitive than all the members of Chenopodiaceae in having 2-several ovules instead of a solitary ovule. A common ancestory for the Amaranthaceae and Chenopodiaceae near Phytolaccaceae is visualized by Cronquist (1981). In the past, a direct evolutionary line was proposed from the Phytolaccaceae to Chenopodiaceae and Amaranthaceae through *Microtea* and *Lophiocarpus* (two phytolaccaceae genera). But evidence from palynology (Skvarla and Nowicke, 1976) and ultrastructure of sieve element plastids (Behnke, 1974), however, prove that *Lophiocarpus* is not at all related to the Chenopodiaceae or Amaranthaceae. In the case of *Microtea* the affinity is not yet clearly established.

The Cactaceae and Aizoaceae share a number of characters such as succulent nature of the plant body, CAM photosynthetic pathway and tricolpate pollen grains. The Phytolaccaceae and Nyctaginaceae containing starch grains along with proteins in their sieve element plastids (Behnke, 1976). The lack of anomalous secondary growth bring the Portulacaceae and Basellaceae together. Production of anthocyanins instead of betalains is the binding character of Molluginaceae and Caryophyllaceae.

The monophyletic origin of Caryophyllales is evident from morphological, embryological, palynological, cytological and features of ultrastructure of sieve element plastids characters, and the data obtained from rRNA-DNA hybridization also support~~s~~ this concept. The 16S rRNA-DNA homology percentage within betalain families are within the range of 100-92% whereas in between betalains and anthocyanin families it is 90-83%. But in the case of some other families such as Bataceae (a non-pigmented family which was formerly included in Caryophyllales) the percentage of homology was less than 75% (Chang and Mabry, 1973). Serological data also exhibit a more or less similar pattern (Jensen, 1965).

The origin of Caryophyllales from Magnoliales of the subclass Magnolidae is proposed by Cronquist (1968) and Baxbaum (1961). Within the Magnoliales the family Illiceaceae (now in the order Illiceales (Cronquist, 1981)) with a single genus *Illiceum* possess the apocarpous pistil expected of an ancestral group of Caryophyllales. But the specialized embryological and chemical characters of Caryophyllales make it difficult to consider Illiceaceae or any other related group of Magnolidae as an ancestral group to Caryophyllales (Ehrendorfer, 1976).

The Centrifugal development of stamens and the apocarpous pistil in both Dillinedae and Caryophyllales is often cited as an evidence of close alliance existing between these two groups of plants. (Alismatales is the only group other than these two taxa with centrifugal development of stamens in Angiosperms).

The presence of apocarpous pistil in Ranunculales prompted Hallier (1912) and Takhtajan (1966) to propose the Ranunculales as a possible ancestral group of the Caryophyllales. The presence of some ~~of~~ similarities between the pollens of some Caryophyllales and Ranunculaceae (Cronquist, personal communication) is significant in this context.

Mabry (1974; 1977) suggested a common ancestral line originating from the proangiosperms which subsequently gave rise to two lines prior to the origin of floral pigments.

Though the Caryophyllales are considered a natural monophyletic order, differences of opinion exist about the placement, evolutionary tendencies and intrafamilial classification of a number of families. Lot of work is necessary to set the controversies at rest and to add a touch of finesse to the taxonomic treatments of these taxa. Some of the issues which were taken up in the present project are:-

1. <u>The placement of Gisekia</u>:- <u>Gisekia</u>, the most aberrant genus of the Phytolaccaceae, is transitional to the

Molluginaceae and has often been referred to that family. It resembles the Molluginaceae in habit and inflorescence characters and in some characters of Pollen and ovules, but is highly anomalous in that family in having separate carpels and betalains.

2. The concept of primitiveness of the Phytolaccaceae:- The uniovulate carpels of Phytolaccaceae make it difficult to consider them ancestral to the multiovulate Aizoaceae, Cactaceae and Caryophyllaceae.

3. The relationship of the Aizoaceae to Cactaceae:- The relationships of these two families (which are mostly leaf succulents) which are similar in ecological specializations and distribution are assessed.

4. Position of the Cactaceae in the Caryophyllales and the Primitiveness of leafy Pereskia:- The validity of the position of the Cactaceae in Caryophyllales and the primitiveness of leafy Pereskia are evaluated. The origin of Cactaceae from Phytolaccaceae (which is accepted in the absence of any other alternative) also is verified.

5. Primitiveness of Celosieae:- Primitiveness of the Celosieae which prevents deriving the Amnranthaceae from the Chenopodiaceae.

6. The status of the Basellaceae:- The status of the Basellaceae as a separate family evolved from the Chenopodiaceae. The unusual nature of the Basellaceae and Protulacaceae in Caryophyllales

due to the absence of anomalous secondary growth and the inter-relationships between these two families.

7. Origin of Caryophyllales from Illiceales/Ranunculales:- The validity of the assumptions of the Illiceales/ Ranunculales to be the procaryophyllalean group.

8. The Placement of Polygonaceae:- The placement of Polygonaceae, which some authors (Hallier, 1912) kept in Caryophyllales.

9. Position of Petiveria:- The position of Petiveria, which was placed in Phytolaccaceae.

10. The identity of the Illecebraceae:- The identity of Illecebraceae which was considered as a subfamily of Caryophyllaceae.

11. The Placement and relationships of Sphenoclea:- The pa placement and relationships of Sphenoclea which is kept in Phytolaccaceae by Airy shaw (1948).

12. The tribal and subfamilial classification of families:- In addition the tribal and subfamilial classification of families such as Amaranthaceae (3 tribes, 3/2 subfamilies), Chenopodiaceae (11 tribes, 3/2 subfamilies), Phytolaccaceae (3/5 tribes, 4/5 subfamilies), Caryophyllaceae (3 tribes, 2/3 subfamilies), Cactaceae (2 tribes, 3 subfamilies) and Polygonaceae (6 tribes, 3 subfamilies).

The Caryophyllales are a large group with world wide distribution. Chemical work already available pertain mostly to the European Species, very few of the Asian Species are studied Chemotaxonomically. This work it is believed, would supplement the already available data and would help in the understanding of the group.

In the present study leaf phenolics (flavones, flavonols, glycoflavones and Phenolic acids) of 98 plants, belonging to the Amaranthaceae (23), Chenopodiaceae (13), Cactaceae (9), Portulacaceae (3), Nyctaginaceae (4), Phytolaccaceae (4), Basellaceae (1), Caryophyllaceae (24) and Polygonaceae (17), have been studied in detail. The presence or absence of tannins, proanthocyanidins, iridoids, steroids, alkaloids and quinones are other chemical markers used.

The work on Caryophyllalean taxa is presented family wise. The assessment of the interrelationships of the families is done at the end. In addition, a Cladistic analysis involving the morphological and chemical characters is attempted to construct a Phylogenetic tree of the Caryophyllales.