CHAPTER I

INTRODUCTION

Acanthaceae is a large tropical family of about 250 genera and 2,500-2,700 species with opposite leaves: in the mesophyll or epidermal cells and parenchyma of the axis occur cystoliths.[1,2] Quite a number of plants in the family are used in the Tropics in medicine.[2]

Barleria Linn. is a considerable genus of the family Acanthaceae and many of its members are reputed for their medicinal values in many parts of the world.

The genus comprises approximately 180 species of undershrubs or shrubs, distributed in the warmer parts of the world.[3] They are chiefly plants of rather dry climates.[4] As well as medicinal consideration, some are cultivated as ornamental hedge-plants, for the sake of their pretty flowers.[3]

In India, of about the occurring 30 species, the followings are considered medicinal: *Barleria buxifolia* Linn., *Barleria courtallica* Nees, *Barleria cristata* Linn., *Barleria longiflora* Linn., *Barleria prionitis* Linn. and *Barleria strigosa* Willd. The leaves and roots are used for cough and in inflamations.[3]

There are several species of *Barleria* in Africa and many of them are used for medicinal purposes. It was reported that

Barleria macrostegia Nees is taken by a Tswana tribe woman about the time of the climacteric; and the root decoction of *Barleria ovata* E. Mey. ex Nees is used by the Zulu tribe for the relief of a condition characterized by painful nodules under the skin.[5] A root decoction of *Barleria prionitis* Linn. is used by the Shambala tribe as a mouth wash to relieve toothache.[6] The Masai tribe use a decoction of the stem and root of *Barleria mucronata* Lindau as an emetic, and a root decoction of *Barleria cephalophora* Lindau for the relief of muscular pain.[6]

In Malaysia, Indonesia and the Philippines, the species of *Barleria* are not abundunt, but even so some of them are used as domestic medicine such as *Barleria cristata* Linn.,[7] *Barleria prionitis* Linn.[4.7.8] and *Barleria lupulina* Lindl.[4]

Only six species of *Barleria* are native to Thailand and another one is an exotic plant. They are *Barleria biloba* Imlay, *Barleria longiflora* Linn., *Barleria siamensis* Craib, *Barleria cristata* Linn., *Barleria lupulina* Lindl., *Barleria strigosa* Willd. and *Barleria prionitis* Linn. respectively.[9,10] The latter four species are used for folk medicine in various purposes.[11]

<u>*Barleria cristata* Linn.</u>

Synonyms: *Barleria alba* Lodd.[12]
Barleria caerulea Herb. Lamb.[12]
Barleria chegosa Herb. Madr.[12]
Barleria ciliata Roxb.[7]
Barleria dichotoma Roxb.[12]

Barleria indica Desf.[12]
Barleria laciniata Wall.[13]
Barleria lactea Desf.[12]
Barleria napalensis Nees[13]
Barleria nuda Nees[12]
Barleria prinoides Hort. Monsp.[12]
Barleria venulosa Nees[12]
Barreliera cristata Blanco(7)
Ruellia ciliata Wall.[12]

Local names:

Angkaap "อังกาบ" (Central)[9]
Angkaap muang "อังกาบม่วง" (Centrāl)[10,14]
Angkaap kaanphluu "อังกาบกานพลู" (Northern)[9]
Kaan chang "ก้านชั่ง" (Chiengmai)[9]
Khan chang "คันชั่ง" (Tak)[9]
Luem thoa yai "ลืมเผ่าใหญ่" (Chiengmai)[9]
Thong ra-aa "ทองระอา" (Bangkok)[9]

Barleria cristata Linn. is a small, erect, much-branched shrub, commonly found in hedges in India, Malaysia, Indonesia and the Philippines.[4,7,15] It occurs also in China[7] and Burma.[13]

In Thailand the plant grows wild in forests, brushwood, grass-field, and is cultivated here and there as a garden ornamental. The roots are used medicinally as a diuretic and as an emmenagogue.[11]

In India the seeds are supposed to be an antidote for snake bite; the roots and leaves are used to reduce swellings; and an infusion of the roots and leaves is given in coughs.[16,17]

According to Dhar *et al.*,[18] the alcoholic extract of the entire plant *Barleria cristata* Linn. was found to have hypoglycaemic

activity in albino rats. Bhakuni *et al.*[19] reported that the extract obtained from *Barleria cristata* var. *dichotoma* showed central nervous system depressant activity and produced hypothermia in mice.

Barleria lupulina Lindl.

Local names: Salet phangphon "เสลดพังพอน" (Central)[9]
Chong ra-aa "ชองระอา" (Trad)[11]
Chek che kiam "เช็กเชเกี่ยม" (Chinese)[9]
Phimsen ton "พิมเสนต้น" (Central)[9]

Barleria lupulina Lindl. is a small spiny bush, often cultivated for medicinal and ornamental purposes. The branches and leaves have a considerable reputation for curing snake-bites.[4,11] The root is used with spiritous liquor as an application against all venoms, including poisonous snakes. A paste of pounded leaves is applied with benefit to boils and glandular swellings, and when mixed with spiritous liquor is applied as an emollient poultice in urticaria.[11]

It was recorded that a Siamese, who had been bitten several times by cobras, and always relied on his preparation from this *Barleria* to cure him, was bitten by a hamadryad, and as he kept his alcoholic extract handy he swallowed some and applied it to the wound. This did not save him, but it was well authenticated that he lived much longer, after being bitten, than sufferers usually do.[20]

Barleria lupulina Lindl. is also recorded to be medicinal in Malaysia, as a cure for toothache and as a remedy for snake-bites. It is used in hedges in Java, or Indonesia; and is medicinal, but in what

way is not recorded.[4]

Barleria prionitis Linn.

Synonyms: *Barleria bispinosa* Hochst.[12]
Barleria brevispina R. Br.[12]
Barleria diacantha Hochst.[12]
Barleria echinata St. Lag.[12]
Barleria hypocrateriformis Hochst. ex T. Anders.[12]
Barleria hystrix Linn.[7]
Barleria pubiflora Benth.[13]
Barleria quadrispinosa Strokes[12]
Barleria spicata Roxb.[12]
Barreliera prionitis Blanco[7]
Justicia appressa Forsk.[12]
Prionitis hystrix Miq.[7]
Prionitis pubiflora Miq.[12]

Local names:
Angkaap nuu "อังกาบหนู" (Central)[9]
Khieo kaeo "เขียวแก้ว" (Central)[9]
Khieo nuea "เขียวเนื้อ" (Central)[9]
Mankai "มันไก่" (Northern)[9]

Barleria prionitis Linn., the exotic species of Thailand, is a small, stiff, prickly plant,[4] commonly cultivated in low hedges.[3] It is found throughout tropical Africa and Asia, and is recorded to be medicinal in various countries.[4]

In Thailand the roots are used to prepare a febrifuge.[4,11] The plant is also considered as a febrifuge in Abyssinia and Indonesia.[7]

The Shambala tribe in Africa used a root decoction of the plant as a mouth wash to relieve toothache.[6] In Indonesia the roots are finely ground, mixed with lemon juice, and applied to ringworm.[8] In India a paste of the roots is applied to boils and glandular swellings.[3]

In Malaysia the juice of the leaves is given for indigestion with constipation; and a poultice of the leaves is used for congestion of the liver.[4]

In Indonesia the leaves are chewed for toothache. A poultice of the leaves is used for ringworm and rheumatism.[4] The plant is also used as a diuretic. It has a high content of potassium but no important organic substances.[21] Boorsma[22] criticized that the diuretic action may be due to the potassium content.

In the Philippines a decoction of the leaves and stem-tops is used for bathing in cases of febrile catarrh.[7,8] The juice of the leaves, administered in a little honey or with sugar and water, is a favourite medicine in the catarrhal affections of children accompanied with fever and much phlegm and is applied to bleeding gums. The juice of the leaves, applied to the feet in the rainy season, prevents their cracking or laceration. It is also dropped into the ear in otitis.[7]

In India the leaf is used for aphthae and catarrh, against intermittent fever, paralysis, rheumatics, liver diseases, jaundice and dropsy.[5] The dried bark is given in whooping cough, and the juice of the fresh bark with milk, in anasarca.[7,17] Medicated oil is applied to

unhealthy wounds.[23] Chopra *et al.*[21] included the plant in a group having antiseptic properties.

Spencer *et al.*[24] reported that the plant had given negative antimalarial test.

Gujral *et al.*[25] reported that a decoction of leaves of *Barleria prionitis* Linn. in a dose of 2.5 ml/100 gm, was found to have moderate diuretic activity in rats compared to urea as the standard drug.

Taneja and Tiwari[26] reported that the plant contains iridoid glycosides which are Barlerin, Acetylbarlerin and other three unidentified iridoids.

Datta ans Biswas[27] had pharmacognostically studied of *Barleria prionitis* Linn. and reported that the leaf has pairs of epidermal cells containing calcium carbonate crystals. The caryophyllaceous type stomata on lower and upper surfaces have stomatal indices of 23.70 and 0.69 respectively, the palisade ratio of 8.10 and the vein-islet number 9.

Barleria strigosa Willd.

Synonyms: *Barleria caerulea* Roxb.[13]
Barleria macrophylla Heyne[13]
Pseudobarleria caerulea Oerst.[13]

Local names:
Sangkoranee "สังกรณี" (Central)[9]
Jukkarohinee "จุกโรหินี" (Chonburi)[11]
Kamphaeng yai "กำแพงใหญ่" (Loei)[9]

Kheefai nokkhum "ขี้ไฟนกคุ้ม" (Prachin Buri)[9]
Saam-sib gleeb " สามสิบกลีบ" (Pak Thong Chai, Nakorn Raja-see-ma)[14]
Thoeng-dee "เทิ่งดี" (Karen-Kanchanaburi)[9]
Yaa ngon kai "หญ้าหงอนไก่" (Northern)[9]
Yaa hua naak "หญ้าหัวนาค" (Northern)[9]

Barleria strigosa Willd. is a small, branched-shrub. It grows wild in forests, and is cultivated as a garden ornamental.[11] The plant is also found native to Malaysia, India[13] and Indonesia.[15]

The root of *Barleria strigosa* Willd. is used in severe spasmodic cough in India;[17] and as a febrifuge in Thailand.[11]

Further chemical and pharmacological investigations of these *Barleria* are required. The present work has been undertaken to provide sufficient pharmacognostical study, for the identification of *Barleria cristata* Linn., *Barleria lupulina* Lindl., *Barleria prionitis* Linn. and *Barleria strigosa* Willd. which may be considered helpful for the investigators as well as the consumers to obtain the exact plants.

PURPOSE AND SCOPE OF INVESTIGATION

Four species of the genus *Barleria* (Family Acanthaceae), namely *Barleria cristata* Linn., *Barleria lupulina* Lindl., *Barleria prionitis* Linn. and *Barleria strigosa* Willd. are locally known in Thailand as Angkaap (Angkaap muang), Salet phangphon, Angkaap nuu and Sangkoranee respectively. All of them have long favourable reputation in the treatment of widely different ailments. Since no previous study on the Pharmacognosy of these indigenous medicinal plants have been made with the exception of *Barleria prionitis* Linn.; the present investigation was undertaken with the aim of finding the correct identities of the plants or plant fragments which could be identified and differentiated from other closely related species.

Scopes of Investigation

1. To show the morphology and histology of the plants.
2. To show the microscopic characters of the powder of leaves.
3. To determine the microscopic data on palisade ratio, stomatal index, stomatal number, vein-islet number and veinlet termination number in order to make use of such factors as diagnostic aids for identification of the specimens.
4. To illustrate the patterns of chemical constituents of the leaves by using thin-layer chromatographic facility as a means of identification of drugs.

Definition of Terms Used

Palisade Cells are a type of photosynthetic cells of the mesophyll of a leaf occurring mostly just beneath the upper epidermal surface layer.(28) The cells are elongated and more or less cylindrical and arranged in one or more rather regular, relatively compact layers near the ventral, or upper side of the leaf with the long axis of the cells perpendicular to the leaf surface.(29)

Palisade Ratio is the average number of palisade cells beneath each upper epidermal cell.(30) It is obtained by counting the total number of palisade cells beneath four contiguous upper epidermal cells and dividing the number by four.(31)

Stomata are the openings in the epidermis through which gaseous interchange takes place between the intercellular spaces of the subepidermal cells and the atmosphere. These openings are spaces between two specialized cells known as guard cells. The term 'stoma' is also applied to the opening in the epidermis plus the surrounding guard and subsidiary cells.(29) In the latter case the term 'stomatal apparatus' is preferred.

Stomatal Number is the average number of stomata per square millimetre of epidermis. In recording results the range as well as the average value should be recorded for each surface of the leaf and the ratio between the two surfaces.(30)

Stomatal Index is the percentage proportion of the ultimate divisions of the epidermis of a leaf which have been converted into stomata.(30)

$$\text{Stomatal Index} = \frac{S}{E+S} \times 100$$

Where S = number of stomata per unit area

and E = number of ordinary epidermal cells in the same unit area.

Vein-Islet is the minute area of photosynthetic tissue encircled by the ultimate divisions of the conducting strands of a leaf.(30)

Vein-Islet Number is the number of vein-islets per square millimetre of leaf surface calculated from four contiguous square millimetres in the central part of the lamina, midway between the midrib and the margin. The result should be given to the nearest 0.5(30)

A Veinlet Termination is a small vein-tip running out from the surrounding veinlets into the centre of each vein-islet.(32) It is the ultimate free termination of a veinlet or branch of a veinlet.(30)

Veinlet Termination Number is the number of veinlet terminations per square millimetre of leaf surface.(30,33)

SURVEY OF LITERATURES

Palisade Ratio

The mesophyll of a leaf comprises the palisade and spongy parenchyma in proportion related to plant species and habitat.[28] The palisade parenchyma appears to play a major role in photosynthesis of the leaf[34] and its occurrence is of diagnostic value.[32]

The palisade cells are cylindrical, closely packed in one or more layers with their long axes at right angles to the epidermis, and usually found on the adaxial surface of the leaf.[32,34] In some plants, such as *Thymelaea*, the palisade parenchyma is found only on the abaxial side of the leaf.[34] In xerophytes the palisade tissue often occurs on both sides of the leaf, with the spongy tissue much reduced or absent.[28] The similar arrangement also occurs in the bifacial leaves, such as *Eucalyptus globulus* Lab.[32] Leaves with relatively undifferentiated mesophyll, as found in many hydrophytes, have no palisade tissue.[28]

The development of the palisade tissue depends largely upon light intensity. There may, therefore, be great variation in the proportion and arrangement of the palisade parenchyma in the same species growing under different conditions.[29] However, it is evidently found that the palisade cells of the mesophyll bear a definite relation to the epidermal cells and the palisade ratio is sufficiently constant to serve as a diagnostic character of species.[32]

The determination of palisade ratio was first introduced by Zornig and Weiss in their studies of the Compositae in 1925. They stated that the average number of palisade cells beneath an upper epidermal cell was of diagnostic value. Although the number of palisade cells per unit area increased successively from the base of the leaf to the apex, but since there was a corresponding diminution in the area of the epidermal cells, the ratio remained almost constant.(35)

Until in 1933, Wallis and Dewar introduced the term "palisade ratio" as a figure obtained by counting the total number of palisade cells beneath four upper epidermal cells and dividing the number by four. They investigated the palisade ratios of different varieties of buchu and found it possible to distinguish *Barosma pulchella* Bartl. & Wendl., *Barosma venusta* Eckl. & Zeyh., *Barosma ovata* Bartl. & Wendl., and *Barosma peglerae* Dümmer from *Barosma betulina* (Thunb.) Bartl. & Wendl.(36)

Wallis and Forsdike, in their investigation of the palisade ratio of *Atropa belladonna* Linn., *Scopolia corniolica* Jacquin and *Solanum nigrum* Linn., found that the palisade ratio did not change with the age of the leaf, the habitat of the plant, or from year to year within either of these species.(37) Dewar found the palisade ratio useful in differentiating between the leaves of some species of *Digitalis*;(38,39,40) and Feinstein and Slama found it useful in distinguishing leaves of *Atropa belladonna* Linn. from *Datura stramonium* Linn., *Digitalis purpurea* Linn. and *Hyoscyamus niger* Linn.(31)

George determined the palisade ratio ranges for the upper and lower epidermises of Alexandrian and Tinnevelly Senna leaflets. He showed that the lower epidermis of *Cassia acutifolia* Delile. and the upper epidermis of *Cassia angustifolia* Vahl. both have palisade ratios very near 7.5, that the upper epidermis of *Cassia acutifolia* Delile. has a palisade ratio of 9.5 and the lower epidermis of *Cassia angustifolia* Vahl. has a palisade ratio of 5.0, and that the identity of a powder of either species of Senna can be established from the mean of 20 to 30 palisade ratio determinations on epidermal fragments, a value above 7.5 indicating *Cassia acutifolia* Delile. and a value below, *Cassia angustifolia* Vahl.(41)

The number of layers of palisade is also useful in certain plants, e.g., *Cassia montana* Heyne, sometimes found as an adulterant of Senna, has 3 or 4 layers of cells in the palisade tissue, whereas Senna has only one layer of palisade cells.(32)

Stomatal Index and Stomatal Number

Stomata are a type of epidermal structure possessing great diagnostic value.(32) They are apertures in the epidermis, each bounded by two specialized cells termed the "guard cells" which are usually accompanied by two or more distinct cells known as subsidiary, or accessory cells. These subsidiary cells appear to be associated functionally with the guard cells and their arrangement neighbouring to the guard cells is the basis of types classification of the stomata.(28,34)

Functionally, stomata are of the greatest importance since it is through these openings that gaseous interchange between the intercellular space systems and the outer air takes place. Upon this diffusion through the stomata, the functions of respiration, transpiration, and photosynthesis largely depend.(29)

The stomata are most common on green aerial parts of plants particularly the leaves. In green leaves they occur either on both surfaces or on one only, either the upper or more commonly the lower.(28) The number of stomata per square millimetre of epidermis is different in different plants. It is apparently related to the humidity of the environment; variations in amount of light seem to have no effect.(42) The density of stomata has been established as 100 to 300 per square millimetre for leaves of many species.(43)

The investigations of Timmerman indicated that stomatal number varies considerably with the age of the leaf, thus the actual number of stomata per square millimetre is variable for the same plant if records are made for different years.(44) It is also indicated that stomatal numbers are usually useless for distinguishing between closely allied species, but that in certain cases the ratio between the number of stomata on the two surfaces may be of diagnostic importance, as it is possible, for example, to distinguish *Datura inoxia* Mill. from other species of *Datura*.(45)

In 1925, Salisbury indicated that the number of stomata increases toward the apex and margin of the leaf where the cells decrease in size,

the proportion of stomata to epidermal cells remaining the same.[42] He also showed that a high correlation coefficient exists between the number of stomata and the number of epidermal cells per unit area of leaf surface of a given species. He proposed the following formula for the calculation of the stomatal index:

$$\text{Stomatal Index} = \frac{S}{E+S} \times 100$$

where S is the number of stomata per unit area and E is the number of ordinary epidermal cells in the same unit area. The stomatal index expresses the percentage proportions of the ultimate divisions of the epidermis of a leaf which have been converted into stomata.[31]

Whilst stomatal number varies considerably with the age of the leaf, stomatal index is highly constant for a given species and may be determined on either entire or powdered samples.[30] Rowson[46] and Forsdike[47] showed that stomatal index values may be used to distinguish between leaves of co-generic species, such as leaflets of Indian from those of Alexandrian senna and also leaves of *Atropa belladonna* Linn. from those of *Atropa acuminata* Royle ex Lindl.

Stomatal index is employed in the European Pharmacopoeia, 1969, to distinguish leaflets of Indian and Alexandrian sennas.[30]

Vein-Islet Number

Vein-islet number is the number of vein-islets per square millimetre of leaf surface calculated from four contiguous square millimetres in the central part of the lamina, midway between the midrib

and the margin. The result should be given to the nearest 0.5.[30]

In a leaf, single or several closely associated vascular bundles form the veins of which the arrangement imparts a characteristic appearance to leaves.[28,34] Either in the reticulate or parallel venation, the veins anastomose to one another.[28] The branching and anastomosis of these veins subdivide the mesophyll into small portions, called "vein-islets", which are the smallest areas of the mesophyll bounded by the thinnest branches of the vascular bundles, and in a way represent more or less well-defined photosynthetic units.[29,34]

The vein-islets usually contain small vein-tips, or terminal vein-endings, running out from the surrounding veinlets into the centre of each islet; but in some plants the vein-islets may lack free vein-endings.[34]

The size and shape of the vein-islets vary with different types of venation and with different species. In some plants, especially in the ferns and grasses, definite islets do not exist.[29] It has been shown that the number of vein-islets per unit area of leaf surface is constant for any given species of plant and can be used as a character for the identification of species.[32]

The term "vein-islet" was first introduced in 1915 by Benedict, who defined it as the size of aggregation of photosynthetically active cells surrounding by the veinlets. He suggested that the average vein-islet area is of physiological significance.[48]

According to Ensign[49] and Levin,[50] the vein-islets increase in size as the leaf matures, their growth being a part of the general growth throughout the leaf. In full growth leaf of any one species, the number of vein-islets per unit area of leaf surface is apparently fairly constant, regardless of the size of the leaf or the age of the individual plant.[50]

Levin determined the vein-islet numbers of a number of species of senna, coca, digitalis and buchu leaves and indicated that the vein-islet number frequently serves to distinguish closely related plants, as in the case of the *Barosma* species. It will be noted that *Barosma serratifolia* (Curt.) Willd. and *Barosma bathii* Dümmer, which cannot be distinguished from *Barosma betulina* (Thunb.) Bartl. & Wendl. by their palisade ratios, are distinguished from the official leaves by their vein-islet numbers.[51]

The shape of the vein-islets is also frequently characteristic and will often enable one to sort out a mixture of leaves which have been broken into small fragments.[32] Forsdike[47] showed that the appearance of leaf venation under a hand lens, when viewed by transmitted, and by reflected light, may be used to distinguish medicinal leaves from their common adulterants.

Veinlet Termination Number

The ultimate divisions of the vascular bundles in leaves terminate in what are known as veinlet terminations,[30] or terminal vein-endings,[34] or vein endings,[28] or bundle ends.[28,29]

In leaves that have more or less definite vein-islets, the veinlet terminations bend into the mesophyll of the islet and end abruptly near its centre. Some veinlet terminations may be somewhat enlarged at the tip or branched in various ways. In leaves with parallel veins, for example, the leaves of grasses, or in leaves without definite vein-islets, the veinlet terminations may be merely short spurs from the veins extending into the mesophyll.(29)

The structure of veinlet terminations varies somewhat with the species. Probably in mesophytic dicotyledons the veinlet termination commonly consists of a single spiral or reticulate xylem element accompanied by a single specialized cell; the two elements surrounded by the parenchymatous bundle sheath of which the extensions also have a conducting function in the leaf. The veinlet terminations of hydathodes and some glands, especially the glands of insectivorous plants which function in digestion, have more elaborate structure than typical veinlet terminations.(29)

The veinlet terminations and the small veins forming the vein-islets supply the mesophyll with water and nutrients and absorb and remove the products of photosynthesis. Because of the distribution of the vein-islets and veinlet terminations the distance through which water and materials in solution travel through the mesophyll is always about the same.(29) In the apple leaf, for example, the distance between veinlet terminations or between veinlet terminations and veins bordering the vein-islets is about 88 micra.(52)

The degree of branching of these vein-endings differs in the leaves of different plants. Thus, for instance, in the leaves of *Euphobia* or *Ricinus*, very many such blind ends may be found in a single vein-islet, in *Morus* there are somewhat fewer, in *Quercus boissieri* Reut. very few, and in the leaves of *Quercus calliprinos* Webb blind vein-endings, or veinlet terminations, are absent or almost so.(34)

The number of veinlet terminations per unit area of leaf surface is of diagnostic value in certain cases. Hall and Melville who defined the term "veinlet termination" as the ultimate free termination of a veinlet or branch of a veinlet, suggested that the number of veinlet terminations per square millimetre of leaf surface may be used to differentiate coarse powders of certain leaves belonging to co-generic species. They have found veinlet termination number useful in distinguishing between Peruvian and Bolivian coca leaves and between Alexandrian and Tinnevelly senna leaflets.(33) They also indicated that the veinlet termination number is not significantly dependent on the position at which it is determined.(53)

Thin-Layer Chromatography

Chromatography is an analytical method for the purification and separation of organic and inorganic substances. It is particularly useful for the fractionation of complex mixtures, the isolation of unstable substances and the separation of closely related compounds (isomers, homologues, etc.).(54) The original method of chromatography was described in 1906 by Tswett, who used it for the separation of coloured substances,(55) and the name chromatography stems from this.(56)

However, the limitation of coloured compounds no longer obtains, and most chromatographic separations are nowadays performed on mixtures of colourless substances, including gases.(56)

Besides an important role in the separation and purification of mixtures of substances, various techniques of chromatography also serve as diagnostic tools in determining the number of components in a system and to learn what they are, if possible, without actually isolating them. Still the quantitative determination of each component can be done by means of chromatography.(57)

The feature common to all chromatographic methods is the use of two phases, one 'stationary' and the other 'mobile'. Separations depend on the relative movement of these two phases and on the fact that the substances to be separated distribute themselves between the mobile and the stationary phases in proportions which vary from one substance to another.(56)

Chromatographic methods may be classified according to the nature of the stationary phase, which may be a solid or a liquid. If the stationary phase is a solid the method is known as 'adsorption' chromatography; if a liquid, as 'partition' chromatography. In each case the mobile phase may be either a liquid or a gas.(56)

In general, adsorption chromatography involves a relatively non-polar moving phase and works best when the substances to be separated are not very polar. The major advantages over partition chromatography are that larger quantities can be separated in comparable systems and

that a controlled temparature is not necessary. Partition chromatography, on the other hand, generally involves polar solvents and mixtures of very polar compounds such as carbohydrates or amino acids; and since it is basically dependent upon the distribution coefficients of the substances in question, which are, in turn, highly sensitive to temparature and other conditions, a carefully controlled atmosphere is required.(57)

Since the inception of chromatography as a column technique discovered by Tswett in 1906, the principal landmarks in its progress have been its virtual rediscovery in 1930, the invention of synthetic ion-exchange resin in 1935, the introduction of paper chromatography in the early 1940's, and finally the development of gas-solid and gas-liquid chromatography in the late 1940's and early 1950's. Subsequent expansion in the use of chromatographic methods has been rapid and continuous, with the result that a substantial volumn of literature on the subject has appeared, dealing not only with particular separations but also in much specific detailed with improvements in technique.(56)

The first major development in diagnostic chromatography was the paper chromatography pioneered by Consden, Gordon and Martin in 1944(58) and 1947.(59) The method was fantastically successful and was rapidly adopted all over the world and in every type of laboratory. The major advantages of paper chromatography are its extreme simplicity and the fact that relatively inexpensive equipment is needed. However, since it is a partition technique with the stationary liquid phase held

on a piece of paper, it works best with polar developers and small amounts of polar substances.[57]

The second, primarily diagnostic technique of chromatography was gas chromatography. This was either a partition chromatography as pioneered by Martin and Singe in 1941,[60] or an adsorption chromatography as pioneered by Turner in 1943.[61] The advantages of the method are its speed of operation, its almost unbelievable degree of resolution and the fact that the results can be interpreted quantitatively. The disadvantages are that relatively expensive and complex equipment is needed and that the substances to be separated must have at least some vapour pressure at workable temparatures. Although hampered by technical difficulties, the use of gas chromatography as a preparative technique has been quite successful.[57]

Thus it would appear that a diagnostic technique which is adsorptive in nature and which combines the technical simplicity of paper chromatography and the speed of gas chromatography is lacking. Ideally, such a method should lend itself to quantitative interpretation and should be useful also as a preparative method. Most of the conditions are fulfilled by thin-layer chromatography. The method was concieved as early as 1938 and developed largely during the early 1950's. However, it has been used more extensively since about 1958 and now appears to be in an era of rapid development and almost universal adoption.[57]

In essence, thin-layer chromatography is a type of adsorption chromatography where the adsorbent is a thin layer of some solid deposited on a glass plate support. In operation, it is analogous to paper chromatography. The substance to be separated is placed a short distance from one end of the layer and is resolved by a solvent passing through the layer by capillary action. The development is carried out in a simple closed system as in paper chromatography, but is much more rapid. When the proper solvent mixtures are used, the method can become a partition technique. In quantitative assay, the result obtained involves the errors of 3 to 5 per cent. Thin-layer chromatography shows promise as a preparative means for quantities of one gram or less.(57)

Thin-layer chromatography was developed because of a specific need for a rapid method which would separate small amounts of compounds. Soon after its development it was apparent that the method was more than a micromethod for separating compounds. It was means of (1) investigating adsorbents and solvents for column work, (2) following the course of elution chromatography of colourless compounds, (3) checking the course of reactions and (4) carrying out certain reactions such as oxidations, reductions, dehydrations and so forth, directly on the strip or plate. By these means and other reactions applied directly to the unknowns, an insight into the type of compound could be gained. All these functions of thin-layer chromatography are enhanced by the speed of the method and the fact that only minute amounts of material are needed.(57)

The basic principle of thin-layer chromatography was described by two Russian authors, Izmailov and Shraiber, in 1938. They dusted aluminum oxide onto glass plates and separated various substances on these loose layers. They also applied the method to the separation and characterization of extracts of medicinal plants.[62] The method was later developed by Meinhard and Hall in 1949. They employed a binding agent (starch) to give the layers greater mechanical stability.[63] Kirchner *et al.*[64] developed the procedure further and demonstrated its applicability to the separation and identification of terpenes. Whereas these authors worked with narrow strips of glass ("chromatostrips"), Reitsema[65] used wider glass plates ("chromatoplates") on which several samples could be chromatographed side by side or two-dimensional chromatograms could be run.

Although a few compounds other than terpenes were separated, the general scope of thin-layer chromatography was not recognized, until Stahl[66,67,68] standized the procedure in 1958 and showed its wide applicability. In the paper of Stahl,[67-71] thin-layer chromatography was first introduced as a procedure for analytical adsorption chromatography. He described an ingenious and practicable device for preparing layers ("open columns") about 250 micron thick, of a special adsorbent "Kieselgel G" (silica gel with a plaster of Paris binder) available from E. Merck., A.G., Darmstadt, Germany.[67]

Thin-layer chromatography has considerable advantages over paper chromatography for the separation of lipophilic substances. Recently the method has also been applied to the separation of hydrophilic

compounds and has been systematically compared with paper chromatography for types of compounds (protein and nucleic acid components). It was shown that if suitable materials were used for the layer the method was as effective as paper chromatography or - in separations of amino acids or nucleotides - superior to it.(66)

The most important advantages of thin-layer chromatography are excellent sharpness of separation, high sensitivity, and great speed. Many a separation that requires many hours on paper can be accomplished in a few minutes on a suitable layer.(66) It is possible to use more drastic reagents (such as concentrated sulphuric acid) on thin layer than would be permissible on paper. On the other hand, R_f values are not so reproducible as they are in paper separations. The variations are probably attributable to the difficulty of getting an absolutely uniform and reproducible thickness in the thin layer.(56)

Thin-layer chromatography is today an indispensable standard method in many laboratories because of its selectivity, sensitivity, and rapidity. It is replacing paper chromatography to an increasing extent. Separations have been made possible which could be accomplished with paper chromatography only with great difficulty, or not at all.(66)

Among the various applications in chemistry, medicine, biology, and pharmacy thin-layer chromatography can be used to determine characteristic component-patterns for drugs, plant extracts and biochemical preparations. The comparison of these with patterns of adulterated or diseased samples can provide interesting results.

Such techniques have been used to study "chemical races" in plants, drug contaminants, mint oils and lipid components of multiple sclerosis patients.(57)

Two-Dimensional Thin-Layer Chromatography

Two-dimensional development is particularly valuable for mixtures of many components.(77) Sometimes, more components are in a mixture than will separate clearly on a one-dimensional strip. Or, perhaps, the mixture contains substances that differ from one another so much that two solvent systems should be used. In these situations chromatography is often carried out in two directions which are perpendicular to one another. Such procedure is called "two-dimensional chromatography". It has been used extensively in thin-layer chromatography.(57)

The general procedure of two-dimensional thin-layer chromatography is as follows. The sample is spotted in one corner of a square layer and developed firstly in direction 1 with solvent 1. The plate is then removed from the tank and dried. The substances are partly separated and fall along a line near one edge of the plate. The plate is now placed in a second solvent so that the previously formed component spots along the bottom of the plate are again submitted to chromatography. The components that were not resolved in the first direction are now separated.(57,66)

The major point of variation in this technique is what is done to the solvent system or to the layer between the two developments.(57)

In order to obtain reproducible results, the layer must always be treated in exactly the same way before development in the second direction. Thus, for instance, the conditions of the intermediate drying must never be altered.(66)

The advantage of two-dimensional thin-layer chromatography, other than its excellent sharpness of separation and rapidity, is that the detection sensitivity is considerably greater than on a two-dimensional paper chromatogram.(66)

Chemistry of the Acanthaceae

Chemistry of the Acanthaceae has not been studied thoroughly by the chemists. Lists of some plant constituents prepared by Gibbs,(1) Hegnauer and Kooiman,(72) and some others are as follows:

1. Carbohydrates.

Maltose	from	*Asteracantha longifolia* Nees(5)
		Hygrophila sp.(1)
Glucose	"	*Blepharis edulis* Pers.(17)

2. Calcium Oxalate Crystals.

Needle-crystals are common; but in certain species bundles of bodies which are usually described as acicular fibres, but which resemble large raphides are reported to be found and are stated to be peculiar to this family.(1)

3. Calcium Carbonate Crystals.

Cystoliths are commonly found in this family. They occur in the mesophyll or epidermal cells of the leaves, and in the parenchyma of the roots and stems.[2,75]

4. Saponins.

Saponins are reported present or probable present in:

Aphelandra sp.[1]
Strobilanthes roseus Nees[1]

Saponins are reported absent or probable absent in:

Adhatoda[1]
Aphelandra[1]
Asystasia[1]
Blepharis[17]
Brillantaisia[1]
Carlowrightia[1]
Crossandra[1]
Dianthera[1]
Dicliptera[1]
Jacobinia[1]
Justicia[1]
Lepidagathis[1]
Mendoncia[1]
Pseuderanthemum[1]
Sanchezia[1]

5. Tannins.

Tannins are reported present in:

Acanthus mollis Linn.[1]
Daedalacanthus nervosus T. Anders.[1]
Mendoncia sp.[1]
Pseuderanthemum sp.[1]
Ruellia devosiana Morr.[1]
Sanchezia nobilis Hook. f.[1]
Strobilanthes roseus Nees[1]

Tannins are reported absent in:

Aphelandra[1]
Asystasia[1]
Beloperone[1]
Blepharis[1]
Brillantaisia[1]
Crossandra[1]
Dianthera[1]
Jacobinia[1]
Mackaya[1]

6. Seed-Fats.

Arachidic acid	from	*Adhatoda vasica* Nees[73]
Behenic acid	"	*Adhatoda vasica* Nees[73]
Cerotic acid	"	*Adhatoda vasica* Nees[73]
Lignoceric acid	"	*Adhatoda vasica* Nees[73]
Linoleic acid	"	*Adhatoda vasica* Nees[73]
		Asteracantha longifolia Nees[5]
		Blepharis edulis Pers.[1]
		Hygrophila spinosa T. Anders.[1]
Myristic acid	"	*Asteracantha longifolia* Nees[5]
Oleic acid	"	*Adhatoda vasica* Nees[73]
		Asteracantha longifolia Nees[5]
		Blepharis edulis Pers.[1]
		Hygrophila spinosa T. Anders.[1]
Palmitic acid	"	*Asteracantha longifolia* Nees[5]
		Blepharis edulis Pers.[1]
		Hygrophila spinosa T. Anders.[1]
Stearic acid	"	*Asteracantha longifolia* Nees[5]
		Blepharis edulis Pers.[1]
		Hygrophila spinosa T. Anders.[1]

7. Cyanogenetic Glycosides.

Cyanogenesis is reported in:

Blechum brownei Juss.[1]	(leaves, root)
Daedalacanthus nervosus T. Anders.[1]	(shoot)
Graptophyllum hortense Nees[1]	(leaves, bark, flower)
Hemigraphis strigosa Villar[1]	(stem, root)
Odontonema schomburkiana Kuntze[1]	(shoot)
Strobilanthes roseus Nees[1]	(shoot)

HCN is failed to be found in:

Acanthus eminens C. B. Clarke[1]	(shoot)
Acanthus spinosus Linn.[1]	(leaves)
Adhatoda vasica Nees[1]	(shoot)
Anisacanthus virgularis Nees[1]	(shoot)
Aphelandra aurantiaca Lindl.[1]	(shoot)
Asystasia travancorica Bedd.[1]	(shoot)
Barleria cristata Linn.[1]	(shoot)
Barleria prionitis Linn.[1]	(shoot)
Barleria strigosa Willd.[1]	(shoot)
Beloperone angustiflora Stapf[1]	(shoot)
Beloperone guttata Brandegee[1]	(shoot)
Beloperone oblongata Lindl.[1]	(shoot)
Brillantaisia lamium Benth.[1]	(shoot)
Brillantaisia palisotii Lindau[1]	(shoot)
Crabbea reticulata C. B. Clarke[1]	(shoot)
Crossandra infundibuliformis Nees[1]	(shoot)
Crossandra massaica Mildbr.[1]	(shoot)
Crossandra pungens Lindau[1]	(shoot)
Dicliptera tweediana Nees[1]	(shoot)
Eranthemum igneum Lind.[1]	(shoot)
Eranthemum lindaui C. B. Clarke[1]	(shoot)
Fittonia verschaffeltii Coem.[1]	(shoot)
Graptophyllum hortense Nees[1]	(shoot)
Hygrophila gigas Burkill[1]	(shoot)
Hygrophila spinosa T. Anders.[1]	(shoot)
Hypoëstes phyllostachya Baker[1]	(shoot)
Jacobinia coccinea Hiern.[1]	(shoot)
Jacobinia pauciflora Benth. & Hook.[1]	(leaves)
Jacobinia pohliana var. *velutina* Hort.[1]	(shoot)
Jacobinia suberecta Andre.[1]	(leaves)
Justicia carnea Hook.[1]	(shoot)
Justicia extensa T. Anders.[1]	(shoot)
Lankesteria barteri Hook. f.[1]	(shoot)

Mackaya bella Harvey[1]	(shoot)
Nelsonia canescens Spreng.[1]	(shoot)
Petalidium canescens C. B. Clarke[1]	(leaves)
Petalidium spp.[1]	(shoot)
Pseuderanthemum spp.[1]	(shoot)
Ruellia devosiana Morr.[1]	(shoot)
Ruellia graeciscans Backer[1]	(shoot)
Ruellia macrantha Mart.[1]	(shoot)
Sanchezia nobilis Hook. f.[1]	(shoot)
Schaueria calycotricha Nees[1]	(shoot)
Strobilanthes anisophyllus T. Anders.[1]	(shoot)
Strobilanthes atropurpureus Nees[1]	(seed leaves)
Strobilanthes glutinosus Nees[1]	(shoot)
Strobilanthes isophyllus T. Anders.[1]	(shoot)
Strobilanthes pentstemonoides T. Anders.[1]	(shoot)

8. Mucilage.

Mucilage was observed in: *Asteracantha longifolia* Nees[1]
Pseuderanthemum spp.[1]

9. Allantoin.

Allantoin is recorded from *Blepharis*.[1]

10. Coumarins.

Coumarin from *Peristrophe*[1]
Rhinacanthus[1]

11. Isocoumarins.

Isocoumarins are reported present in *Blepharis*.[1]

12. 6-Hydroxy-flavones.

6-Hydroxy-flavones are reported from *Andrographis*.[1]

13. Chalcones.

Chalcones are reported from *Asystasia*.[1]

14. Lignans.

Diphyllin-methyl ether is reported from *Justicia*.[1]

15. Leucoanthocyanins.

Leucoanthocyanins are reported present in:

Graptophyllum hortense Nees[1]
Mackaya bella Harvey[1]
Pseuderanthemum sp.[1]

Leucoanthocyanins are reported absent in:

Acanthus spinosus Linn.[1]
Aphelandra tetragona Nees[1]
Asystasia coromandeliana Nees[1]
Barleria cristata Linn.[1]
Barleria strigosa Willd.[1]
Beloperone guttata Brandegee[1]
Crossandra sp.[1]
Daedalacanthus nervosus T. Anders.[1]
Fittonia argyroneura Coem.[1]
Jacobinia magnifica Benth. & Hook.[1]
Jacobinia suberecta Andre.[1]
Peristrophe speciosa Nees[1]
Ruellia dipteracanthus Hemsl.[1]
Ruellia graeciscans Backer[1]
Strobilanthes anisophyllus T. Anders.[1]
Strobilanthes roseus Nees[1]

16. Orobanchin.

Orobanchin is reported present.[1]

17. Indigo.

Indigo is reported from:

Amphiscopia sp.(1)
Leptostachya sp.(1)
Nelsonia sp.(1)
Strobilanthes flaccidifolius Nees(76)

18. Resins.

Rhinacanthin	from	*Rhinacanthus nasutus* Kurz(77)
Some soft resins	"	*Acanthus ilicifolius* Linn.(78)
Neutral and acid resins	"	*Barleria prionitis* Linn.(78)

19. Iridoids (Aucubin-type glycosides).

Acetylbarlerin	from	*Barleria prionitis* Linn.(26) (leaves)
Barlerin	"	*Barleria prionitis* Linn.(26) (leaves)
Cardanthera-Pseudoindican	"	*Cardanthera triflora* Ham.(74) (leaves)
Catalpol	"	*Mackaya bella* Harvey(74) (leaves)
Catalpolester	"	*Mackaya bella* Harvey(74) (leaves)
Unidentified Iridoids	"	*Acanthus* (2 species)(74) (2 in seeds, none in leaves)
"	"	*Andrographis* (3 species)(74) (1-3 in seeds)
"	"	*Anisacanthus* (4 species)(74) (2-4 in seeds)
"	"	*Aphelandra* (1 species)(74) (1 in seeds)
"	"	*Barleria prionitis* Linn.(26) (3 in leaves)
"	"	*Barleria* (6 species)(74) (several in seeds and leaves)

Unidentified Iridoids	from	*Brillantaisia* (2 species)[74] (1 in seeds)
"	"	*Chamaeranthemum* (3 species)[74] (1 in seeds)
"	"	*Crossandra* (6 species)[74] (1-3 in seeds)
"	"	*Dicliptera* (1 species)[74] (seeds)
"	"	*Duvernoia* (1 species)[74] (seeds)
"	"	*Ecbolium* (1 species)[74] (1 in seeds)
"	"	*Eranthemum* (3 species)[74] (2 in seeds)
"	"	*Hemigraphis* (2 species)[74] (2 in seeds)
"	"	*Hygrophila* (5 species)[74] (1 in seeds)
"	"	*Hypoëstes* (2 species)[74] (seeds and leaves)
"	"	*Mackaya bella* Harvey[74] (1 in leaves)
"	"	*Pachystachys* (1 species)[74] (seeds)
"	"	*Peristrophe* (2 species)[74] (seeds)
"	"	*Petalidium* (2 species)[74] (2-3 in seeds)
"	"	*Pseuderanthemum* (2 species)[74] (several in seeds)
"	"	*Rhinacanthus* (1 species)[74] (seeds and leaves)
"	"	*Ruspolia* (1 species)[74] (1 in seeds)
"	"	*Schaueria* (1 species)[74] (1 in seeds)

Iridoids are reported absent in:

Blepharis (1 species)[1]	(seeds)
Crabbea (1 species)[1]	(seeds)
Daedalacanthus (2 species)[1]	(seeds)
Dipteracanthus (1 species)[1]	(seeds)
Dyschoriste (2 species)[1]	(seeds)
Elytraria (3 species)[1]	(seeds and leaves)
Geissomeria (1 species)[1]	(seeds)
Ruellia (11 species)[1]	(seeds and leaves)
Strobilanthes (2 species)[1]	(seeds)

20. Potassium Salts.

Potassium salts are reported rich in:

Andrographis paniculata Nees[11]	(plant)
Asteracantha longifolia Nees[17]	(seeds)
Asystasia gangetica T. Anders.[77]	(plant)
Barleria prionitis Linn.[22]	(plant)
Hemigraphis colorata Hall. f.[22]	(plant)
Hygrophila salicifolia Nees[73]	(leaves)
Rhinacanthus nasutus Kurz[7]	(plant)

21. Lactones.

Andrographolide is reported present in:
Andrographis paniculata Nees[17]

Andrographolide is reported absent in:
Andrographis echioides Nees[17]

22. Anthraquinone Glycosides.

Oxymethylanthraquinone from *Rhinacanthus nasutus* Kurz[3]

23. Alkaloids.

Justicine	from	*Justicia gendarussa* Burm. f.[77]
Vasicine	"	*Adhatoda vasica* Nees[8]
Quinazolines	"	*Anisotes* sp.[1]
Quinolines	"	*Macrorungia* sp.[1]
A purine alkaloid	"	*Asteracantha longifolia* Nees[5]
A bitter alkaloid	"	*Acanthus ilicifolius* Linn.[78]
		Hygrophila salicifolia Nees[73] (seeds)
		Justicia procumbens Linn.[79]
Traces of toxic alkaloid	"	*Asystasia gangetica* T. Anders.[77]

24. Sterols.

Hygrosterol	from	*Asteracantha longifolia* Nees[5] (roots)
		Hygrophila sp.[1]
Sitosterol	"	*Adhatoda vasica* Nees[73] (seeds)
Unidentified sterols	"	*Asystasia gangetica* T. Anders.[11]

25. Diterpenes.

Diterpenes are reported present in *Andrographis*.[1]

26. Triterpenoid Saponins and/or Sapogenins.

The compounds are reported from *Asteracantha*.[1]

27. Other Triterpenes.

Other triterpenes are reported from *Andrographis*.[1]