MORPHOLOGICAL & PRELIMINARY PHYTOCHEMICAL STUDIES OF DRUG YIELDING HERBS

DISSERTATION

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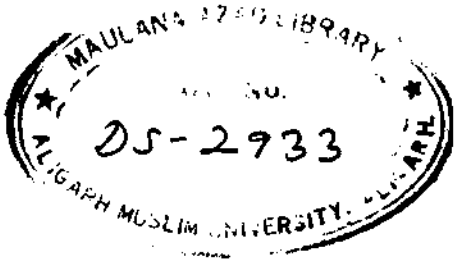

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In the Name of Allah, the Beneficent, the Merciful

And He it is who sends down water from the could, then We bring forth with it buds of all (plants), then We bring forth from it green (foliage) from which We produce grain piled up (in the ear); and of the palm-tree, of the sheaths of it, come forth clusters (of dates) within reach, and gardens of grapes and olives and pomegranates, alike and unlike; behold the fruit of it when it yields the fruit and the ripening of it; most surely there are signs in this for a people who believe.

SURAH AL-ANAM (Verse 100)

Dedicated

to my

Beloved Husband

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CERTIFICATE

This is to certify that the dissertation entitled **"Morphological and Preliminary Phytochemical Studies of Drug Yielding Herbs"**, submitted to the Aligarh Muslim University, Aligarh is a faithful record of the work carried out by **Mrs. Regina** in partial fulfilment of the requirements for the award of the degree of Master of Philosophy.

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Professor

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INTRODUCTION

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Our country is rich in vegetation and most of the herbs and other plants of medicinal value are found all over the country. Hence they form the most important part of native materia medica. There exists a large number of plants which grow wild and wither away without being evaluated.

Drugs which are used medicinally today are either obtained from nature or of synthetic origin. Natural drugs are obtained from three natural sources viz- plants, animals and minerals.

As my project deals with pharmacognosy and phytochemical studies, thus first of all I will give a brief idea about that.

Pharmacognosy may be defined as the scientific study of those substances which are used or have been used in medicine and pharmacy. The word "pharmacognosy" was coined in 1815 by C.A. Seydler. The word pharmacognosy is formed from two Greek words, pharmakon means drug and gnosis means knowledge. Thus pharmacognosy has also been defined as "Simultaneous application of various scientific disciplines with the

object of acquiring knowledge of drugs from every point of view". It may also be expressed as an applied science that deals with biological, biochemical, therapeutic & economic features of natural drugs and their constituents.

Phytochemical study deals with the chemical substances of organic nature which are found in plants through the activity of their individual cells. The process by which the plants are able to convert the simple chemical substances into complex organic compounds with the help of enzymes is known as biosynthesis. The medicinal value of any plant drug depends on the nature of the chemical constituent present in it and is referred to as active principle.

The main groups of phyto constituents of therapeutic significance can be classified as, carbohydrates glycosides, tannins and phenolic compounds, lipids, volatile oils and resins, resin combination and alkaloids.

HISTORY OF MEDICINAL PLANTS

India has a long tradition of the use of drugs derived from plants and herbs in Ayurveda and Unani Tibb

system of medicine. A persual of ancient literature like "Charaka", Susurta" & Avicenna shows that there is much information available in them based on a reasonably strong foundation of scientific methodology available at that time.

The crude drugs which have been used in the past and the present are many in number and cannot be easily distinguished by morphological characters. In case of vegetable crude drugs the identification is generally followed by anatomical studies. Anatomical features have become more important in identification of crude drugs, as these drugs form mostly isolated parts of the plants such as bark, pieces of stem, root, leaf or fruit etc and not the whole plant. Under such isolated conditions of different plant parts they could only be identified on the basis of their structure.

Medicinal plants in vogue from time immemorial are being used in prevention and cure of diseases and for promotion of health. The medicinal value of drug plant is due to the presence of some chemical substances in the plant tissues that produce a definite physiological action on human body. The most important of these substances are the alkaloids, compounds of carbon, hydrogen, oxygen and nitrogen, glycosides,

essential oils, fatty oils, resins, mucilages, tannins and gums. Some of these materials are powerful poisons and therefore the preparation and administration of the drugs should be left entirely in the hands of skilled pharmacists and physicians.

Panini (7th century B.C.) derived the word "Dravya" meaning drug from the nomid rest "Dru", meaning plant. The uses of several plant drugs are mentioned even in vedic age. The Rishis residing in hermitages in the forests seen to have shown interest in the study of the properties of the medicinal herbs for the sake of humanity and used them according to their needs. They had gathered information regarding poisonous and non-poisonous characters of the plants from primitive forest dwellers in addition to the knowledge of herbal medicines they gradually acquired.

The Aryans had rudimentary knowledge of medicines when they came to this country "Oshadi Suktem" of Rigveda, the oldest repository of Indian wisdom is, perhaps the oldest scientific account of classification of medicinal plants. A systematic and detailed account of medicinal plants is given in "Charaka samhita" (1000 B.C.).

History of Unani (Greco-Arabian) system of medicine can be traced to ancient times (131-200 A.D.). The Unani or Greco-Arab medicine historically owes its origin to Greek philosopher physicians. It was through a chain of developments till perfected during the ascendency of Muslim civilization in Asia and Africa. However, the muslims continued to call it Unani (I-onian) medicine, thereby gratefully acknowledging its

Greek origin. The European historians, on the other hand, call it Arab medicine.

Unani medicine is a 'wholistic' medicine and does not confirm Knowledge to any particular branch of it but refers to knowledge as a total recognition of the condition of the person presented to the physicians. It subscribes to the definition of medicine given by Avicenna that it is a branch of knowledge which deals with the state of health and/or disease in the human body and also for the purpose of adopting suitable measures for preserving or restoring health.

The Unani system of medicine is based upon the pythagorean theory of four proximate qualities and/or the Hippocratic theory or four humours. The four

qualities are: hot, cold, moist and dry. These are the qualities felt by human sense of touch and/or perception and their consequential effect on human physique and the life in general.

The four pythagorean qualities or states are referred to the living beings and the human body. Their material representatives are the four elements which are to be taken as simple or elementary constituents of the human body. These are earth (representing solids), water (representing liquids) air (representing gases) & fire (representing heat of the body). Of the four states or qualities referred above, two are active viz hot and cold; and two passive that is moist and dry. The two former act upon the two later which may reciprocate.

The humoural theory of Unani medicine is that humours are the components of the fluids of the body. They are formulated and happen to be four in number viz- blood, phlelgm, yellow bile and black bile. They are all contained in the blood; the red part of it is blood; the white, phlegm; the yellow, yellow bile and the dark, the black bile or melancholic. According to the Unani system, health is primarily that state in which these constituent substances are in balanced proportion to

each other, both in strength and quality, and operate through co-ordination. A Unani physician attaches great importance to diet and digestion, both in health and disease.

In Unani medicine great emphasis is laid on madicatrix nature (viz tabiat, mudabhara, badan), i.e. the natural faculty inherent in human system to correct any accidental fault or deficiency. It is the power which restores the disturbed equilibrium of human system. It is a sort of built in extra capacity in human body which meets the demands in emergencies and in conditions when normal channels are obstructed. A Unani physician has to activate this power and ensure that it does not fail before the climax of the disease.

Ayurveda is much popular among Indians, as the medicines of these systems not only provide cure for diseases, but are also safe and free from side effects. In this system mainly bark, stem, roots, seeds, leaves & flowers are used as a source of medicine. It is said that Acharya Vagbhata (900 A.D.) one of the eminent Ayurvedic physicians lived somewhere in Kerela and taught eight disciples known as "Ashta Vaidyans". It is

regarded that physicians of Kerala are the followers of Vagbhata.

This ancient system which is based on nature was very popular in all parts of Kerela. But after invasion by the Britishers the modern i.e. Allopathy system enjoyed more Government partronage and the people gradually started preferring this system. Added to this, the introduction of abstract medicine in the form of basic chemicals & the demonstration method of pharmaceutical for bringing back quick relief in sufferings was instant admiration and popularity. This charm of allopathy was reinforced by rapid advance made in pharmaceutical sciences all over the European countries which brought out new discoveries of sulpha, synthetics drugs and antibiotics. This swept away all other systems of medicine and consequently the practice of Indigenous system of medicine slowly receded into the country side. Ayurveda & Unani Tibb system did not make serious attempts to utilize the modern tools of analytical chemistry, instrumental technique and st andarization of potency of drug into its system. The lack of exposure of later day physicians to science of Botany and pharmacognosy made them dependent on herb collectors and dealers which consequently caused

deterioration in the efficacy of preparations. The slow progress in these disciplines made Ayurveda and Unani to lessen its popularity and patronage and consequently resulted in attracting low investment both in research and drug production. But recently popularity of Ayurveda and Unani Tibb system popularity has grown in the west due to serious side effects of modern allopathic drugs and now a time has come back and western countries are showing special interest in purchasing Ayurvedic and Unani products. Therefore looking the requirements of local population and of the west world it has become necessary to concentrate on our old traditional Ayurveda Unani system of therapy.

A survey of literature has revealed that some details regarding pharmacognostical studies of plant selected in the present study have been carried out by several workers in the past. However, none of them has attempted to probe the morphological and preliminary phytochemical details of the species which have been presently selected for investigation.

PLAN OF STUDY

PLAN OF STUDY

To study the xylem, phloem (Bark), leaf & root drugs in the selected species of medicinally important plants, the following aspects will be worked out.

(A) MICROSCOPIC STUDY OF XYLEM

(a) Primary xylem

(b) Secondary xylem

(i) Size & shape of tracheary elements.
(ii) Variation with age.
(iii) Types of rays.
(iv) Quantitative study of the constituent tissue.
(v) Preliminary phytochemistry.

(B) MORPHOLOGICAL STUDY OF BARK

(i) Macroscopic study:

(a) Origin from trunk, branches or roots whole bark or inner part only.

(b) Size & shape.

(c) Outer surface - lenticels, cracks or furrows, colour before and after scraping.

(d) Inner surface, colour, striation, furrows.

(ii) Microscopic study

The bark will be studied in transverse, tangential and longitudinal sections to find out the composition & spatial relationship of the different tissue components of bark such as fibres, sclereids, phloem parenchyma, ray cells and periderm. The bark used

for drug is mostly in dry form, therefore the vital elements like sieve tube and companion cells cannot be used as mark of identification. So only sclereids and fibres and their amount and distribution pattern are of common use in morphological studies.

(C) STUDY OF LEAF

(i) Variation in the orientation of leaf
(ii) Shape of the leaf
(iii) Margin of the leaf
(iv) Texture of the leaf
(v) Petiole of the leaf
(vi) Venation pattern
(vii) Arrangement of the areole
(viii) Shape of the areole
(ix) Size of the areole.

Anatomical studies

(i) Anatomy of the petiole.
(ii) Anatomy of leaf blade.
(iii) Epidermal cells (shape, size & number)
(v) Epidermal trichomes
(iv) Stomata

(a) Types of stomata

(b) Size of stomata

(c) Stomatal index

(v) Mesophyll cells
(vi) Vascular bundles
(vii) Secretory ducts
(viii) Stone cells
(ix) Cell contents
(x) Substances in the form of crystals
(xi) Venation
(xii) Determination of vein islet number
(xiii) Determination of vein termination number
(xiv) Determination of palisade satio

(D) STUDY OF ROOT

(i) Primary structure of roots
(ii) Arrangement and number of primary vascular bundles and type of roots.
(iii) Initiation of periderm
(iv) Formation of lateral roots.
(v) Structure of secondary xylem & phloem as seen in T.S. & L.S.
(vi) Structure and dimensional variations
(vii) Tracheary elements and other associated cells.

(E) HISTOCHEMICAL STUDIES

Histochemical test of the drug will be conducted with different reagents.

(F) PRELIMINARY PHYTOCHEMICAL STUDIES

(a) Determination of physical constants

(i) Total Ash Value.
(ii) Acid Insoluble ash.
(iii) Water soluble ash.

(b) Preliminary phytochemical investigations

(c) Fluorescence characters under ultra voilet light.

REVIEW OF LITERATURE

REVIEW OF LITERATURE

THE XYLEM

The vascular system of plant is composed of xylem which is the principal water conducting tissue. The term xylem was introduced by Nageli (1858) and is derived from the Greek xylos, meaning wood (Esau 1965 a). Xylem is the complex tissue consisting of several different types of cells living and non-living. The most characteristic components are tracheary elements which are the non living cells that are pincipally concerned with the conduction of water and minerals and which also to a certain degree have a supporting function. The xylem also commonly contains specialized supporting elements, the fibres, the living parenchyma cells concerned with various vital activities. Fibres may retain their protoplasts in the conducting xylem and thus combine vital functions, as starch storage, with the mechanical one of support (Esau 1965a) Sometimes sclereids may also be present in xylem. In certain groups of plants, the xylem may contain resin ducts. (Fahn, 1974).

Most of the components of xylem have a thick secondary wall, which make things mechanically strong and efficient for the function, which they have to perform. A

similar phenomenon was also observed by Sinnott and Bloch (1945) who studied the development of tracheary elements from parenchyma cells during regeneration of vascular bundles of Coleus. Several workers have suggested that golgi bodies are involved in the formation of the wall thickenings (Wooding and Northcote, 1965). Findings of the others also indicate that the endoplasmic reticulum may also be concerned with this process (Fahn, 1974).

It has been observed (Pickett - Heaps, 1968) that precedding the formation of the wall thickening of the tracheary element micro tubules become grouped in bands between small, regular currugations in the wall. In the later stages many vesicles, probably derived from golgi bodies collect at the regions occupied by the microtubules, which are often seen in cytoplasm close to cell surface may govern cytoplasmic streaming or exert an influence over the disposition of cell wall material (Cronshaw, 1965; Ledbelter and Porter, 1963), and are oriented in the same direction as the cellulose micro fibrils of the developing wall.

After the completion of the elaborated secondary wall thickening and their lignification the protoplast rapidly disorganises. The mitochondria and the golgi

bodies appear to be the last organelles present in the peripheral cytoplams. At the end of the differentiation the remnant of the dead cytoplasm may form an inner lining of the tracheary elements (Czaninski, 1968).

TRACHEARY ELEMENTS

The term tracheary elements is derived from "trachea", a name originally applied to certain primary xylem elements resembling insect trachea (Esau, 1961). Two fundamental types of tracheary elements occur in the xylem, tracheids and vessel elements. In the mature state both are more or less elongated cells (some vessels may be drum-shaped), having lignified secondary walls and containing no protoplast. They differ from each other in that the tracheids are imperforate cells having only pit-pairs on their common walls, where as the vessel members are perforated in certain areas of union with other vessel members. Thus the vessel members are joined into long continuous tubes, the vessel.

The perforation of vessel members commonly occur on the end walls but they may be present on the lateral walls too. The wall bearing the perforation is called the perforation plate. eg. Dysoxylem spectabiles (Patel, 1974), Metrosideros (Sastrapradja and Lemou-reux, 1970),

Nestegis spp. (Patel, 1978) and Paratropis spp. (Patel and Bowles, 1978) or several perforations on their end walls, multiple perforation plate. If the opening in a multiple perforation plate are arranged in a parallel series it is known as scalariform perforation plate eg. Weinmannia spp. (Dickson, 1977), Dillenia spp. (Dickson 1979) or in a reticulate manner - reticulate perforation plate eg. Hymenocallis caribaea when the perforations are almost circular the plate is termed as Forminate perforation plate eg. Ephedra.

PRIMARY XYLEM

Protoxylem and Metaxylem

Protoxylem is the tissue which appears at the begining of vascular differentiation and occupies a characteristic position in the primary vascular system of the plant. The metaxylem, which appears after the protoxylem, is in the process of differentiation while the shoot is elongating and matures after this elongation is finished. The protoxylem elements commonly have annular and spiral thickenings, sometimes also reticulate. The metaxylem may have spiral, reticulate and pitted secondary walls (The Committee on Nomenclature, 1957, limits the metaxylem to the tissue with pitted tracheary elements).

The protophloem elements are narrower then the metaxylem elements but there may be gradual transition in the size of cells between the two parts of the primary xylem (Esau, 1965a).

Secondary Xylem

Usually it is difficult to distinguish between the primary and secondary xylem. The best distinguishing feature between these two tissues is the length of tracheary elements (Bailey, 1944; Khan, 1977). The first formed tracheary elements of secondary xylem are generally much shorter than the tracheary elements of primary xylem. They are even shorter than the pitted tracheary elements of primary xylem which are themselves usually shorter than the spirally thickened elements (Fahn, 1974).

The most distinctive feature characterizing the secondary xylem is the existence of two system of elements which differ in orientation of their longitudnal axis. One system is vertical and the other is horizontal. The vertical system comprises the tracheary elements, fibres and axial parenchyma and the horizontal system, the ray parenchyma. The living cells of the rays and of the axial system are usually interconnected, so that it is possible

to speak of a continuous system of living cells. (Esau, 1965a).

Wood Parenchyma

The axial perenchyma and the ray parenchyma are found in the secondary xylem. The ray parenchyma cells originate from relatively short combial initials - ray initial, while the cells of axial parenchyma develop from fuisform initials. Two types of ray parenchyma cells are found in plants. In the first type, the long axis of the cell is radial, and in the second type it is vertical. The ray parenchyma cells may have secondary walls (Fahn, 1974).

The parenchyma of the xylem serve to store reserve materials such as starch and oil. Tannins, crystals and other substances are also frequently found in many of these cells. A similar trend accumulation of starch and tannins has been reported by Khan (1980) in Callistemon citrinus, Eucalyptus maculata and Eugenia jambolana.

Arrangement of Axial Parenchyma

The amount of axial parenchyma varies in different dicotyledonous species. In some species there is very little axial parenchyma, or it is entirely absent as in

Scaevola (Carlquist, 1970) while in others it constitutes a very large portion of the wood.

There are two basic types of distribution of axial parenchyma, apotracheal in which the parenchyma is typically independent of the vessel, though it may come in contact with them here and there, and paratracheal in which the parenchyma is distinctly associated with the vessels. Both these types are further subdivided. When the apotracheal parenchyma is in the form of small uniseriate strands or single cells scattered irregularly among the fibres, it is said to be diffuse parenchyma. When in cross-section, the axial parenchyma is seen to form concentric bands, it is said to be banded or metatracheal parenchyma eg. Dysoxylum spectablis (Patel, 1974) and Olmedia (Mannega and Borg, 1977). Patel (1974) also observed the occurence of silica inclusions in the chambered parenchyma of Dysoxylum spectablis.

Parenchyma occur at the end of a growth ring in more or less continuous layers, which may be of variable width, are termed terminal parenchyma (Carlquist, 1961). Similar parenchyma formed at the beginning of the growth ring is termed initial parenchyma. For eg., initial parenchyma occurs in ceratonia, Zygophyllum and Spartium.

Patel (1978) also reported the occurrence of initial parenchyma in the members of family oleaceae.

The paratracheal parenchyma also may be variously distributed. If the parenchyma does not form a continous sheath around the vessels as in Acer is said to be scanty paratracheal parenchyma. When the paratracheal parenchyma occurs on one side either external (abaxial) or internal (adaxial) of the vessels it is said to be unilaterally paratracheal parenchyma Parenchyma which forms entire sheaths, of different width around the vessels eg. Tamarix is termed vasicentric parenchyma. In some plants eg. Acacia cyanophylla, Cercis siliquastrum the sheaths in cross section can be seen to have lateral wing-like extensions such parencyma is called aliform parenchyma. In the wood of certain species such as Acacia raddiana and A,albida the aliform parencyma is seen in cross section, to form diagnol or tangential bands; this type of parencyma is termed as confluent parenchyma.

Structure of Rays

The ray usually consists of only parenchyma cells, in dicotyledons. On the basis of the orientiation of the long axis of the cells, as seen in radial longitudinal section, parenchyma cells that form the ray may be of one

type only or of two types. If the ray cells are all elongated in a radial direction i.e., all the cells are procumbent, the ray is homogenous. When the ray in dicotyledonous wood consists of two types of cells, i.e., procumbent and vertically elongated cells (known as upright cells) it is said to be heterogenous (Carlquist, 1961; Jane, 1956).

Heterogenous rays may be uni or multiseriate. The most common type of heterogenous ray is that in which the central position of the ray is multiseriate and consists of the radially elongated cells, while the upper and lower edges contain the vertically elongated cells (fahn, 1974).

The variation in ray structure in different plant species has resulted from divergences during the evolution of xylem (Bailey, 1957; Kribs, 1935).

The decrease in the size of the rays with phylogenetic advancement may have been brought about by changes that took place in the cambium (Barghroorn, 1941). Some of the ray initials may become lost and their place be taken up by fusiform initials of the cambium. A ray may also split as a result of the intrusive growth of the tips of fusiform initials into a group of ray initials. A ray may become split by the changing of some ray intials into

fusiform intials. In this manner aggregate rays are often formed from larger rays. An aggregate ray is defined as a ray comprising a group of small and narrow rays which appear to the unaided eye or under low magnification to be a single large ray (Fahn 1974; Khan 1980).

Distribution of vessels

In dicotyledons, the arrangement of vessels in secondary xylem is characteristic feature and is used in the identification of the species. The vessel arrangement shows two main patterns. When the vessels have essentially equal diameters and are uniformly distributed through a growth ring, the wood is called diffuse porous wood (Esau, 1965a). Examples of species with such woods are Acer spp, Populus alba Acacia cyanophylla and Eucalyptus spp. (Fahn, 1974). Wood with vessels of unequal diameters and with the large vessels localized in early wood are called ring porous for example, Fraxinus, Quercus spp., Robinia pseudoacacia and Pistacia atlantica. Many intermediate forms occur between the two extreme types. Environmental conditions and the age of the plant also influence to a certain extent the arrangement and size of vessels (Khan, 1977; Purkayastha et al 1974).

The development of vessels in early ring porous wood is very rapid and sudden while that in diffuse porous

wood it is slow (Priestly and Scott, 1936). Handley (1936) measured the length of vessels and concluded that the vessels in ring porous wood are larger than those in diffuse porous wood. Several myrtaceae members are found to have diffuse - porous wood such as some species of Eucalyptus (Dadswell, 1972); Psiduim guajava (Khan, 1977); Callistemon cetrinus, Eucalyptus maculata and Eugenia jambolana (Khan 1980) and 12 species of Eucalyptus (Khan et al, 1980)

The radial and tangential diameters of vessels reveal a gradual increase with the increase in girth of the stem axis until a maximum is reached and then a constancy follows in F. infectoria as has been repored by khan (1980) in some myrtaceae members. Khan (1980) has also found the vessel length of Eugenia jambolana to undergo a gradual increase with the increase in the girth of the stem axis till they attain their maximum size and then a constancy is followed.

The woody genera are known that completely lack vessels. These genera belong to the following five families, Chloranthaceae Winteraceae, Tetracentraceae, Trochodendraceae & Monimiaceae (PLANT ANATOMY-Fahn pg-111).

Xylem fibres

Wood fibres are long cells having tapering ends and lignified secondary walls. They vary in size, shape, thickness of the wall and abundance of pitting. The secondary walls of the fibres are often so thick that the lumen of the cells become narrow eg. Talisia (Mennega, 1973). The fibres may be thin walled also. In Paratrophia spp, (Patel and Bowles, 1978) fibres are thin to very thin walled. The fibres of Castilla (Moraceae) are thin walled and wide - lumined (Mennega and Borg, 1977). Irregularly spaced alternate bonds of thin and thick walled fibres are often present in Dysoxylem spectablis (Patel, 1974).

Fibres are divided into two larger groups, xylary fibres and the fibres of the various tissue systems outside the xylem, the extra xylary fibres. These fibres develop from the same meristematic tissues as the other xylem cells and constitute an integral part of the xylem.

Fibres integrade with the imperforate tracheary elements and with the parenchyma cells. Wood fibres are subdivided into two main categories, the fibre tracheids and the libriform - fibres (Committee on Nomenclature, 1957). The fibre tracheids are supposed to be the transitional form between the tracheids and the most specialized libriform fibres. The libriform fibres

resemble phloem fibres (liber = inner bark) and they are usually longer than the tracheids of the plant in which they occur (Fahn, 1974)

The size of wood fibres and fusiform cambial initials from which they are derived were compared in eight species of Eucalyptus. It was found that the wood fibres were 1.5 to 2.3 time longer than the fusiform cambial initials. Growth of the fibres occured usually through the elongation of both the ends of a cell but in three of them 4 - 12% of the fibres exhibit monopolar growth (Khan et al 1979 a) Khan et al (1979 b) reported that the xylem fibres in E. camaldulensis and E. papuana grow intrusively to the extent of about 1.5 - 1.8 times over their mother initials Khan et al (1979, c) have shown that in some verbenaceae the extent of apical growth in xylem fibres varies from 1.8 - 5.4 times over their mother initials in different species.

STORIED AND NON-STORIED WOODS

The xylem derived from a storied cambium may be storied or only partly so, if the original stratification is obscured by changes during the differentiation of the xylem.

When the fusiform initials occur in horizontal tiers with the ends of the cells of one tier appearing at

approximately the same level, such meristem is called storied or stratified cambium. It is characteristic of plants with short fusiform initials. When the fusiform initials in vascular cambium are not arranged in horizontal tiers and their ends overlap, such meristem is termed non-storied or non-stratified cambium. It is common in plants with long fusiform intials. The storied condition is associated with short fusiform initials and is therefore an advance phylogenetic feature (Esau, 1965a).

GROWTH LAYERS

The structural differences between the xylem produced in the early and late wood parts of a growth season is due to the cause of the visibility of the growth layers in the section of wood. The early wood is less dense than the late wood and has generally larger cells and proportionately thin walled. The early wood and the late wood are commonly called "spring wood" and "summer wood" respectively. The early wood of a given season merges more or less gradually with the late wood of the same season, but the division between the late wood of one season and the early wood of the following season is oridinarily sharp. (Esau, 1965a).

THE PHLOEM

The phloem is the principal food conducting tissue of the vascular plants. The phloem and xylem are, as a rule, spatially associated with each other and together constitute the vascular system of plant body. The bark comprises primary phloem, pericycle, cortex & epidermis. Later after the establishment of secondary body, the bark essentially represents secondary phloem and periderm products.

According to Esau (1965), the term bark is applicable to the tissues lying between the epidermis and vascular cambium in dicotyledons and gymnosperms, where periderm formation is of general occurrence. The bark includes three structural zones viz. the conducting and non-conducting phloem, periderm and rhytidome.

STRUCTURE OF PHLOEM

Phloem develops as a peripheral vascular tissue and forms a part of bark. The phloem can be divided into two major types :

(1) Primary phloem

(2) Secondary phloem

(1) PRIMARY PHLOEM

The initiation of the primary phloem starts in

the embryonic stage of the plant and comprises the differentiation after the formation of the primary body. It is derived out of the procambium, the provascular tissue. It consists of the first formed protophloem elements and the later formed metaphloem elements. They are different in structure, development and position.

(a) Protophloem

The position of the phloem that differentiates first has been named "protophloem" (Russow, 1872) Protophloem generally lacks well defined sieve plate and sieve areas and they are not associated with companion cells. (Esau, 1965; Fahn, 1967) However the occurrence of well developed sieve areas on the lateral walls of the protophloem elements was reported by Ghouse et al (1972)

(b) Metaphloem:

The part of phloem which develops after the formation of protophloem & matures after the growth in length of the surrounding tissue is completed is named as "Metaphloem". (Van Tiegham, 1887) In woody & herbaceous species having cambial growth, the metaphloem sieve elements become inactive after the secondary conducting elements differentiate. In such plants the

metaphloem elements may be partly crushed or completely obliterated (Esau, 1965)

(2) **SECONDARY PHLOEM**

The various cell types of secondary phloem develop from vascular cambium. It is generally composed of sieve elements (either sieve cells or sieve tube members, the latter usually with companion cells) phloem parenchyma, phloem fibres & rays. Occasionally sclereids also constitute a part of this system. (Esau, 1965; Ghouse et al 1977; Khan et al; 1976; Khan 1977) Secondary phloem possesses well developed sieve areas and sieve plates, & are arranged in radial files by which it is distinguished from the primary part of the phloem (Esau 1964).

Secondary phloem consists of two main systems the axial or vertical derived from furiform cambial initials and the ray or transverse system derived from ray initials. The elements of axial system are sieve tube members usually with companion cells, axial parenchyma cells, fibres and those of ray systems may contain sclereids, secretory elements of schizogenous & lysogenous origins, laticifers & various idioblasts with specialized contents (Esau, 1965)

In many species of dicotyledons sieve tube members are elongated, bearing mostly compound sieve plates on the inclined end walls eg. Aegle (Bel) Emblica (Amla), Feronia (Kaithbel), Mangifera (Mango), Morus (Mulberry) & Tilia (lime tree), while in others eg. Acer (Maple) & Dalbergia (Shisham) end walls usually bear simple sieve plates. The distribution of fibres is different in various species, Moller, (1882); Strasburger, (1891); Holdheide, (1951); Zahur, (1959); Ghouse & yunus, (1974); Ghouse et al, (1975); Siddiqui et al, (1976); Khan et al, (1978); Ghouse & Jamal, (1978); In some species like Psidium, Duranta Variegata, Vitex negundo, Duranta repens. Fibres are absent (Khan et al, 1977) In Eugenias Jambolana sclereids and fibres together constitute the sclerenchymatous system but did not form any specific pattern of distribution in this zone. (Khan et al, 1982). In Callistemon citrinus the fibre length continues to increasae as the position advances from the cambium towards the periphery of the bark (Khan & Khan, 1983).

On the basis of the structure and functional capacity of sieve tube element the phloem can be divided into 2 categories.

(a) Conducting phloem

(b) Non-conducting phloem

(a) Conducting Phloem :

The phloem is considered to be differentiated into a conducting tissue when the sieve elements become enucleate and develop the other associated specialized characteristics including the conspicuous sieve areas and strands between the cells. Although the active phloem constitutes only a small portion of the bark, it is of outmost importance for the detailed structure of the bark, as all the characters such as shape and length of sieve elements, structure of the sieve plates, companion cells, variation of parenchyma cells etc. are clearly visible in this part of the bark. (Esau, 1964).

(b) Non-Conducting Phloem :

The part of the phloem in which sieve elements have ceased to function is usualy referred as non-conducting phloem. The non-conducting phloem commonly retain parenchyma cells that continue to store starch and tannins until the tissue is isolated from living part of the plants by the activity of phellogen (Esau, 1965). The sieve elements represent the inactive state in various ways. The sieve areas are either covered by a

mass of substance known as callose leading to discarding their function or they get crushed. The identification of non-functioning state of the sieve tube is particularly certain if the elements are more or less collapsed or crushed . In dicolytedons companion cells & some of the parenchyma cells cease to function & even collapse (Esou, 1965) The characteristic of inactive phloem as a whole vary in different plants. In certain dicotyledons Liriodendron (Cheadle & Esau, 1964) Tilia, Populus & Juglans, the shape of non-functioning sieve tube changes slightly (Esau. 1965), While in other like Dalbergia spp.. (Ghouse & Yunus, 1975) some members of Myrtaceae (Khan, 1977) & some deciduous & evergreen tropical species, the sieve elements and their associated cells collapse completely.

In Myrtaceae members the phloem fibres give a characteristic look to the bark structure in transectional view. They are invariably found in aggregates forming fascicles of varying shape & size or in tangentially extended bands. They constitute an area of 15% in C. citrinus, 16% in E. concinna, 28% in E. maculata, 13% in E. microtheca & 20% in E. jambolana in the non conducting phloem (Khan & Khan 1983).

In Psidium guajava L. the non conducting phloem formed the major part of the adult bark and constituted mainly the ray porenchyma, axial parenchyma & sclereids. The axial & ray parenchyma contained varying amounts of ergastic substances such as tannins, starch & calcium oxalate crystals of different shapes & sizes (Khan 1977).

Certain anatomical features such as distribution patterns and amount of sclerenchyma and nature of periderm may be of particular help in the identification of various related species.

(1) **PHLOEM PARENCHYMA** :

In 1858 Nageli reported parenchyma cells in the phloem tissue and called them "bast parenchyma." Esau, 1939 considered parenchyma cells as characteristic feature of the primary and secondary phloem of angiosperms, gymnosperms & cryptogams.

According to Esau (1965) the parenchyma cells are concerned with many of the activities characteristic of living parenchyma cells such as storage of starch, fat and other materials and accumulation of tannins and resins. In the secondary phloem, parenchyma occurs in two systems, the axial and the ray system. The ray

parenchyma constitute the phloem rays (Esau, 1965). Phloem Parenchyma cells may or may not have their origin from the same mother cell as sieve elements. Parenchyma cells are ontogenetically related to sieve elements (cheadle & Esau, 1958; Evert, 1963; Srivastava & bailey, 1962). But recent report of Esau (1970) shows that whether the parenchyma cells are ontogenetically related or not, they do not degenerate when the sieve elements ceases to function.

(2) PHLOEM FIBRES :

The term phloic or phloem fibres is used for fibres originating in primary or secondary phloem. Fibres are important components of phloem, particularly in the secondary tissue (Esau, 1965). They vary considerably in length & generally many times larger than broad. They are elongated elements with tapered ends, narrow lumen and thick secondary walls (Esau, 1965; Fahn; 1967; Cutter, 1969). They are lignified but in some cases they are not. The pits present on their wall are usually simple but sometimes may be slightly bordered (Esau,1965). The number of fibre bands formed in a year differs considerably , depending on the species & age of the shoot. These fibre bands impart a peculiar structure to the phloem which is a

characteristic feature for a genus or species (Holdheide, 1951; Zahur, 1959; Ghouse & Yunus, 1974; Khan et al 1978).

The secondary fibre elements in angiosperms and gymnosperms may occur in continuous or interrupted tangential bands alternating with the bands of remaining elements of phloem. When fibres are few they are usually scattered, singly or in small fascicles among other cells of phloem (Khan et al, 1978; Ghouse et al, 1977; Khan & Khan 1983).

SCLEREIDS

Sclereids are widely distributed in the plant body (De Bary 1884) Sclereids are also components of phloem where they may integrate with fibres Esau (1965) Ghouse et al, (1975) Khan et al (1976). Similarly Khan et al (1976); worked on development & distribution of sclereids in Duranta repens & Nyctanthes arbortristis. Again Khan 1977 worked on the macroscopic & microscopic characters of stem bark of P. guajava & found that mature bark is non fibrous & is characterized by the presence of a variety of crystals of CaO_2, tannins & starch.

Sclereids vary widely in shape size &

characteristics of their walls, crystals are embedaded in the secondary wall of the sclereids in certain species Bailey & Nast (1948), Inamdar & Gangadhan (1974). In some sclereids the deposition of secondary wall is uneven. Sclereids may retain protoplast on reaching maturity otherwise they are dead cells (Esau, 1965).

PHLOEM RAYS

Phloem rays constitute an important part of the horizontal parenchyma of secondary phloem. Usually they are the outward continuation of xylem rays. The manner in which the dilation of rays in non conducting secondary phloem is brought is also considered (Holdheide, 1957; Schneider,1952)

PERIDERM & RHYTIDOME

Periderm is the outer protective tissue of secondary origin Periderm formation is the common phenomenon in stem & roots of dicotyledons & gymnosperms that increase in thickness by secondary growth. The periderm usually consists of following three components viz. The Phellem or Cork produced by the phellogen towards outside, Phellogen or Cork cambium & Phelloderm produced by the phellogen internally.

In most of plants, as soon as the first phellogen ceases to function, second phellogen develops in the tissue below the first one. In this way additional layers of periderm are found in the progressively deeper regions of the stem, thus new phellogen layers arise in deeper regions of the cortex which may exceed even upto secondary phloem. As the phellogen arises in deeper regions and cuts cork cells or phellem towards outside, the living cells outside the phellogen do not get water supply and nutrients & become dead. These dead tissues formed outside the phelloegen constitute the Rhytidome.

In the past a lot of work has been done in relation to "morphological & preliminary phytochemistry of medicinally important plants". A review of important work done in this field is given below:

Jolly (1966) studied the pharmacognostical aspect of the stem bark of Soymida febrifuga A. Jus. which revealed that when the drug analysed for its ash values gave 16.36% total ash 1.5% water soluble ash, 0.23% of acid insoluble and 23.04% of sulphated ash. Similarly the ash values of different barks were studied from pharmacognostical point of view in different plants. e.g. Adhatoda vasica Prasad & Prabhu (1950) Ticomella

undulata Prasad et al (1967) Bombax ceiba Mehra & Karnik (1968), Mimusops elengii Mehra & Raina (1970), Albizzia procera Gupta & Kapoor (1974).

Similarly pharmacognostic studies on the stem bark of Crateava religiosa was done by Chaudhari (1975) and saponin, tannins were isolated from the stem bark. Okada (1976) worked on species of genus Rhamnus. Study on chinese crude drug "xia ku cao" wad done by Namba et al (1976) Pharmacognostic studies of Acorus calamus & its adulterants was done by Dipali et al, (1982) & found that it can be easily identified by its macroscopic & microscopic characteristics. Gupta (1985) worked on stem & root bark of "Dhava" (Anogeissus latifolia) and found that it contained curative properties.

In preliminary screening of 202 plants belonging to 162 genera distributed over 64 natural orders, sources of alkaloids, saponins, sterols, terpene & tannin etc. have been observed by Maiti (1968); Prasad & Jaiswal (1959) showed the presence of alkaloids in the barks of Anthocephalus indicus. The other medicinal plants that had undergone phytochemical scrceening include Albizzia lebbek Shah & Bhattacharya (1960), Acacia augustissima. Hammer & Cole (1964), Pscidia

erythrina Buchi et al (1964), Alstonia venneta Das et al (1965), Zizyphus Zuzuba Memon & Kazi (1967), Cassia fistula Sen & Shukla (1968). Prosopis specigera Khasg et al (1969), Dalbergia latifolia Dhingra et al (1971), Albizzia procera Gupta & Kapoor (1974), Syzygium cumini Bhatia & Bajaj (1975), Simba cuspido Giesbrecht et al (1980). Chemical constituents of Erythrina bark was investigated by Singh et al (1981). Javed (1983) did preliminary phytochemical screening of Salix alba, Ficus benjamina, Jatropha curcas, Jatropha gossipifolia, Legerstroemia speciosa, E. Jambolana, P. guajava, Jasminum grandiflorum, J. sambac.

In pharmacognostical studies the macroscopic & miscroscopic characters of stem bark of different tree species have been described eg. Adhatoda vasica Prasad & Prabhu (1950). Terminalia arjuna & T. tomentosa Shah & Mehta (1956); Melalcuca leucodendron Singh (1956); Ficus glomerata Nayar & Bishtt (1959); Albizzia lebbeck Shah & Bhattacharya (1959 & 1960); Zanthoxylum rhetsa Sukkawala (1961); Piper longum Atal & Banga (1962); Punica granatum Chaudhuri (1963); Ficus benghalensis & F. racemosa Chaudhuri (1965a); Calophyllum inophyllum Chaudhuri (1965b); Sterbulus asper Chaudhuri (1968); Cassia fistula Krishna & Ali (1970); Abroma augusta

Mitra & Prasad (1971). Albizzia procera Gupta & Kapoor (1972); A. lucida Gupta & Kapoor (1973); Cinnamomum Species Karnik (1974); Ficus lacor Mitra & Kapoor (1975); Chinese crude drug Xia-cu-cao" Namba et al (1976); Acorus calamus Dey & Das (1982); Eugenia Jambolana Khan et al (1982); Psidium guajava Khan (1985) Anatomy & pharmacognostic evaluation of the following bark was worked out, Bauhinia purpurea Kumar, Datta (1985), Aminuddin & Girach (1992) worked on indigenous system of medicine of Hemidesmus indicus of family. As clipidaceae, the Calotropis family.

THE LEAF

Turrill (1934) working at Royal Botanical Garden at kew for the first time claimed that morphological features of leaf should not be neglected as it helps in identification as well as in classification of different taxa. Lee (1948) supported the claims of Turril & put forward a system for identification of leaf. Stearn (1956) Krussmann (1960) defined shape categories for leaves. Apical as well as basal shapes were also given more precise formulation. The "Systematic Association Committee for Descriptive Terminology" SADT (1962) gave a new shape.

Hickey (1971 a,b, 1973) made, a great countribution to taxonomy by giving an architectural plan of the dicotyledonous leaves which is based on gross morphology (Turrill, 1950) & venation pattern (Ettingshausen, 1861) of the leaves based on the shape of the whole leaf and of the apex & base. Leaves are seperated into number of categories depending on the course of their principle venation, as most taxa of dicots possess consistent pattern of leaf architecture.

Maedler (1975) went a step further and classified discotyledonous leaves into folia pinnatinervia & folia

palmatinervia. These types were again divided into 10 sub types determined by general leaf morphology of Hickey (1973). Each of these is further sub divided into specific morphological characteristics. Recently, Girardi (1976) utilized different morphological and anatomical features such as margin, texture, position of the petiole, presence of hairs on the leaves, venation patterns and epidermal cells as well as the presence or absence of gland cells for the identification of centain members of Meliacea.

GROSS ANATOMY

1. PETIOLE

The petiolar anatomy has been studied in order to know the vascular supply and its mode of derivation from internode to leaf petiole through the node. The most comprehensive study following an extensive survey of angiospermous taxa was made by Sinnott (1914) who pointed out that reductions and amplifications of trilacunar node resulted in the unilacunar and multilacunar condition.

Bailey & Nast (1944) claimed that in most of the families of vascular plant, combination of uni & tri or

tri & multi lacunar situations were met. Dickson (1970) working on family Dilleniaceae reported that different taxa of this family possess as many as four patterns, unilacunar 1-trace, 2-trace trilacunar & multilacunar. Sehgal & Paliwal (1974) in their study of 150 species of the genus Euphorbia claimed a greater complexity, since a single genus possess three main types of nodal organisation i.e., unilacunar 1-trace, bilacunar 2-trace & trilacunar 3-trace, the last being in majority.

Metcalfe and Chalk (1950) and Howard (1962) Studied the three dimensional vascular patterns of the petiole of woody plants & claimed several features of systematic importance for identification & assessment of the taxa. Later on, Tumanyan (1965) identified three species of Polygonum viz, P. cakatum, P. heterophyllum & P. procumbens on the basis of marked differences in their petiole. Drury (1974) could distinguish 26 species of Australian shrubs and trees belonging to genus Bedfordia, Brachyglottis, Senecio & Traversia with the aid of morphoanatomical characters of the nodes & leaves.

2. LEAF BLADE :

During the last three decades the anatomical

features of the leaves have played an important role in categorizing the natural plant groups and for the elucidation of phylogenetic relationship (Metcalfe, 1956).

The value of leaf anatomy has become well established in taxonomically difficult groups such as the coniferae (Marco, 1931, 1939; Ghouse and Yunus, 1972 a,b) Coniferales and Taxales (Kaushik and Bhattacharya, 1977) and Graminae (Brown 1958; Metcalfe, 1960, 1961; Townrow, 1969) Paunero (1952) had recorded that leaf blade provided sound specifically differential characters in Alopecurus.

Druyts-voets (1971) reported variations in Cyperus & categorized them in 28 sections for identification. Nikolaevskii (1972) made a similar attempt on 200 species pertaining to subfamilies Bambusoideae Ergrostoideae, Fetucoideae and Panicoideae and classified them into chloridoidal, Echino-chloidal and Festucoidal groups. The specific feature of the first group is the preence of 2 facing layers in the vascular bundle (parenchymatous and mechanical) and the diffuse structure of mesophyll. The second type is characterized by the presence of facing layers with crown arrangement

of parenchymatous cells around the bundle and the third type exhibits formation of only one (parenchymatons) layer oriented towards vascular bundle. Yuldashev and Garaeva (1975) studied the leaf anatomy of some species of Gossipium. Nolla (1975) reported the detailed anotomy of Ficus glabra a member of family Moraceae and claimed marked differences in palisade layer, spongy parenchyma and annular collenchyma. It was also compared with other species of the same genus.

3. EPIDERMIS

The epidermis have been given a great deal of attention by botanists since long back. For example, physiologists are concerned with this, as it relates to movements of materials in and out of the plant; pathologists are concerned with the role of epidermis as a barrier to the entrance and exist of disease organisms (Agrios, 1969); ecologists consider it as it relates to adaptation of plants to specific environments while taxonomists and anatomists utilized its characteristics in systematic studies (Carlquist, 1961).

Odell (1932) studying the cuticle in 84 genera of living angiosperms has concluded that non of the epidermal features of the vegetative parts in living and

fossil angiosperms prove worthy to be the basis for diagnosis. Edward (1935) reported that structural differences in the leaf epidermis helped to some extent in distinguishing closely related taxonomic groups. The claims of Cutler (1969) in Aloe species, Robinson (1969) in 102 species of Connellia, Cottendorfia, Navia Schyler (1970) in some Cyperaceae, Pandey (1971) in Cassia spp. Bonger's (1974) in 33 species of Winteraceae, Prakash and Hashim (1974) in 22 species of Adiantum, Singh et al (1975) in 17 species of Ipomoea Srivastava (1975) in some Jasminum are note worthy.

(a) TRICHOMES

The trichomes are epidermal appendages of diverse forms, shapes structure and sizes and are found on almost every part of the plant body i.e. aerial as well as underground (Carlquist 1961). They either persist through out the life of an organ or become ephemeral. They are either meant for protection (Donald, 1973) or play a supporting role by developing sclereids (Guedes, 1976). In the latter case, the epidermis first develops hairs and then it transforms into sclereids. Sometimes they also get modified into glandular hairs, nectaries which produce sugary substances, born on the floral parts (Brown, 1938. Fahn, 1952, 1953; Metcalfe and

Chalk, 1950; Esau, 1965). The trichomes may be classified into different morphological categories (Esau, 1965). The most common type of hairs are uniseriate or multiseriate. The former may be unicellular or multicellular (Kakkar and Paliwal 1974) while the latter are always multicellular and classified into followings :-

(a) Dendroid hair tree like branched

(b) Stellate hair - star shaped

(c) Peltate hair - shield like.

Due to multiple variation in shape, size and the universal occurence of trichomes on the plant body they have been employed for systematic approach as well as in identifying various taxa.

(b) STOMATA

Stomata are minute apertures in the epidermis through which gaseous exchange takes place. Normally these apertures are surrounded by two guard cells. The walls are thickened adjacent to the aperture (Esau, 1965). They differ from other epidermal cells in having unique shape dense cytoplasm, prominent nuclei, chloroplast and starch grains. As reported earlier,

normally two guard cells enclose stomata but single guard cells has also been reported in somehas also been reported in some cases either in degenerating forms (Ahmad 1964 a,b). Stomata are found on almost every part of the aerial plant body (Gupta et al, 1965); Inamdar 1969; Shah and Gopal, 1970) and are more frequent on the leaves and specially on the abaxial side of the bifacial leaf. The non-functional stomata has been reported in submerged aquatic plants like Hydrilla and floral parts viz. petals and stamens of Crinum, Iris, Canna species (Esau, 1965). They are sunken and appear to be suspended in the mesophyll tissue in gymnosperms, in linear rows in monocotyledons (Cyperaceae and Gramineae) and scattered in the dicotyledonous leaves (Paliwal, 1969).

Latter a slightly modified terminology was proposed by solereder (1908) and Metcalf and chalk (1950) which is as follows -

(A) Anomocytic

(Ranunculaceous) - The guard cells remain surrounded by a limited number of indistinguished subsidiary cells.

(B) Paracytic

(Rubiaceous) - The guard cells on either sides

are accompanied with one or more subsidiary cells and adjacent wall is parallel to the long axis of the pore of guard cells while cronquist (1968) defined it as those with two equal subsidiary cell flanking the guard cells.

(C) Anisocytic

(Cruciferous) The guard cells are surrounded by three subsidiary cells out of which one is distinctly smaller than the others.

(D) Diacytic

(Caryophyllaceous) The guard cells are surrounded by a pair of subsidiary cells whose common wall is at right angles to the long aixs.

(E) Actinocytic

In actirocytic type the subsidiary cells are arranged along the radii of a circle.

In gymnosperms Florin (1958) distinguishes two main types of stomatal complex, the haplocheilic (simple lipped), in which the subsidiary cells are perigene, and the syndetochelic (compound lipped) in which the subsidiary cells are mesogene.

In monocotyledons four categories of stomatal complexes have been described (stebbins and Kush, 1961)

two of these have four or more subsidiary cells surrounding the Kidney shaped guard cells (Rhoeo, Commelina) one has two subsidiary cells adjacent to the dumb-bell shaped guard cells (Gramineae) and one has none (Allium). The types with many subsidiary cells are regarded as more primitive.

In the recent past the taxonomic values of stomata have been realized by Paliwal (1965, 1969); Pant (1965) and Payne (1970). The mode of development of stomata, its shape and spatial relations to the neighbouring cells are the features which should be employed in classification and phylogeny was claimed by Florin (1958), Metcalfe and Chalk (1950), Stebbins and Khush (1961); Pant and Bharti (1965) & Paliwal (1969).

VENATION

A leaf may have single vascular bundle as in conifers and Equisetum where as multiveined leaves are common in higher plants (Esau, 1965). In angiosperms two main types of venation pattern have been i.e. the reticulate or netted and the striate or parallel. The former is of wide occurrence among the dicotyledons while the latter is the characteristic of monocotyledons with a few exceptions in both the groups. In some

monocotyledonous e.g. Aracaceae, Orchidaceae and Taccaceae (Schuster, 1910), modified parellel venation which looks like reticulate has been noticed and some dicotyledonous showing parallel venation is in Plantago and Fragopogon (Esau, 1965).

Foster (1950), while studying family winteraceae reported that the careful description of venation together with other details of leaf anatomy can yield valuable taxonomic information. Carlquist (1959) reported in some Hawaiian Asteraceae that minor venation provides taxonomic & evolutionary approaches. It was favoured by Verghese (1969) in the study of several taxa of the family Scrophulariaceae.

Venation pattern, along with epidermal features i.e. hairs & stomata were employed by Girardi (1976) in some Meliaceae in identifying the taxa.

VEIN ISLET & VEIN ENDING

The venation pattern along with numerical relationships to other leaf characters have also been employed by several workers. Schuster (1908) reported that the total length of vein islets in per unit area of leaf surface is a specific character. The term vein

islet was coined by Benedict (1915) & he employed it as a unit of comparision. He concluded that the size of the (vein-islets) at any stage of the leaf growth is a specific character. On the contrary Ensign (1919, 1921) claimed that vein-islets size is not related to the age of the plants.

Fischer (1885) for the first time studied extensively the histology of the vein-endings in dicotyledonous and paid more attention to the nature of phloem in the vein-endings. He categorized his findings into two major groups-principal endings and secondary endings. The former is usually branched structures and always possess phloem tissue and the latter is short, consisting of a single tracheid and devoid of phloem tissues [except in (Cucurbitaceae). The significant feature of Fischers (1885) investigation is that the vein endings normally possess sieve tubes and the phloem like xylem forms a system with free terminal tips.

In the past a lot of work has been done in relation to preliminary phytochemistry & on pharmacognostical aspect of medicinally important plants. Here, some of the important work on leafy drugs have been cited.

The ash values of different leaves were studied from pharmacognostical point of view in different plants eg. Adhatoda vasica (Prasad & Prabhu, 1950); Tecomella undulata (prasad et al., 1967); Bombax ceiba (Mehra & Karnik, 1968); Mimusops elengi (Mehra & Raina, 1970); Varun leaves (Mitra & Kapoor, 1971); Calotropis procera (Gupta et al, 1971); Albizzia procera (Gupta & Kapoor,1974);

In preliminary screening of 202 plants belonging to 162 genera distributed over 64 natural orders, sources of alkaloids, saponin, sterols, terpene & tannin etc., have been observed by Maiti (1968). Shah & Quadry (1968) during the phytochemical studies of leaves of cassia tora & C. occidentalis have formed chrysophenol aloe-emodine, rhein & emodine are found to be present.

Many workers have described the macro scopic and microscopic characters of leaves during the course of pharmacognostical studies of different medicinally important species e.g. Adhatoda vasica (Prasad & Prabhu, 1950); Datura metal (Chaudhuri et al., 1953); Vitex negundu [Madan & Nayar, (1959)]; Calotropis gigentea (Chaudhuri, 1961); Dodonaea viscosa (Sukkawala, 1962);

Vallaris Solanaceae (Madan and Kundu, 1962); Catharanthus roseus & C. pusillus (Chaudhuri, 1963) Cassia sophera (Chaudhuri, 1964); Ocimum spp. (Gupta, 1967) Cassia Tora C. occidentalis (Shah & Quadry, 1968) Calotropis procera (Gupta et al., 1971) Murraya paniculata (Khosa & Prasad, 1972) tejpat (Mitra et al., 1973).

Microscopical studies of the leaves of Eucalyptus globulus & three other species grown in India have been described by Datta & Datta (1952). Solanaceous leaves have been described by Chandra et al, (1972) Chandra (1967) and Singh (1967).

The palisade ratio, vein islet number and stomatal indices were determined in various species e.g. Adhatoda vasica (Prasad & Prabhu, 1950). Datura metal (Chaudhuri, 1953); Vitex, negundo (Madan & Nayar, (1959); Vallaris solanaceae (Madan & Kundu, 1962); Cassia sophera (Chaudhuri, 1964); Ocimum spp. (Gupta, 1967); Calotropis procera (Gupta et al, 1971); Varun leaves (Mitra & Kapoor, 1971).

Babaskin & Barabanov (1980) find out the role of stomata in identifying crude leaves of Ungernia victoris. The form of stomata plays an important role in

identifying crude leaves of Ungernia. The presence of auricle in peristomal cells of the epidermis and the form of epidermal cells proper, apart from the characteristic thickening of the cuticle, in the form of tubercles on the external walls of the epidermal cells are identifying characteristics for distinguishing. Mitra & Yadav (1980) studied the pharmacognostical features of the leaves of M. elengi. (Sapotaceae). Microscopically, the leaf shows a dorsiventral structure with a ranunculaceous or rubiaceous present on the lower surface and striations emanate from the sides of stomata. The powder, when treated with con. HNO_3 turns orange and with 1N NaOH and nitrocellulose in amylacetate emits blackish green fluorescence under UV light. Preliminary phytochemical tests show the presence of sterols, reducing sugars and tannin.

Pharmacognostical study on drug of Thlapsi bursa pastoris Moench (Cruciferae) was done by Varma et al (1981). Diagnostic features include anisocytic stomata on both abaxial and adaxial epidermis. Stomatal index 16.66-25.00; palisade ratio 1-1.5; stellate serrately thick walled trichomes on leaves; each with a wide central circular-pore; sclerenchyma caps above & below vascular bundle in leaves. Sharma & Shome (1983) studied

some of the distinguishing features of Salvia Cubulica Benth.-striated lower epidermis, stomatal ledges broad and smooth; S. lanata Roxb.-abaxial side completely covered over by a thick coat of trichomes; S. macro siphon Boiss.-verucose trichomes with constructed joints; S moorcroftiana wall.-longitudinal folds on basal cells of trichomes; S. officinalis Linn.-curved cylindrical trichomes, cells over veins with characteristic longitudinal ridges, gland stalk very long; S. plebeia R.Br. basal cells of trichomes transversely striated; S. pratensis Linn.-verrucose trichomes and series of irregular folds on lower epidermis; S. spinosa Linn.-Smooth callapsible hairs, folds on general surface similar to S.pratensis. Javed (1983) studied the morphological & preliminary phytochemical studies of leaf samples of some Indian medicinal plants e.g. Salix alba, Ficus benjamina, Jatropha curcas, Jatropha gossipifolia, Legerstroemia speciosa, Eugenia jambolana, Psidium guajava, Jasminum grandiflorum, Jasminum sambac.

Sen & Datta (1984) compared the anatomical features and chemical properties of Nerium indicum (Apocynaceae) and experience of its being admixtured or interchanged with Thevetia peruviana leaf. Well marked

venation pattern, coriaceous texture, sunken stomata multiple epidermis and compact palisade layers on both sides distinguished N. indicum leaf from thevetia leaf. Palisade ratio, crystal frequency, extractive values in different solvents and ash values were lower in Nerium indicum.

Malik & Nawazish (1979) studied the chemical composition of Calotropis procera which he extracted from the leaves. Chemical constituents were determined from dry leaves which have fairly high ash content 10.9 11.7% and high protein content 18.8 - 25.2% where as leaves contain calotropin and calotropagenin. The root bark contain dark brown oil which was characterised and the plant also contains alkaloidal materials.

Birecka et al (1980) extracted high level of unsaturated pyrrolizidine alkaloids while H.indicum contain saturated pyrrolizidines.

El-Azizi et al (1980) extracted ethanolic compound from the leaves of Curatella americana linn. and isolated flavonol glycoside avicularin and gallic acid.

Siddiqui et al (1988) extracted oleanderoic acid and oleanderen from the uncrushed leaves of Nerium oleander; structures of which have been established as

80 Methoxylabdan-18-oic acid, 12-ursene.

Torrenegra et al (1988) extracted 3-oxo-vincadifformine, 14 -hydroxyquebrochamine & the bisindole alkaloid, 14-15 dehydrotetrastachynine from the leaves of Stemmadenia grandiflora.

Irobi & Daramola (1993) studied the antifungal activities of crude extracts of Mitracarpus villosus. Extracts of Mitracarpus villosus leaves were investigated for invitro antifungal activities by agar-diffusion & tube dilution technique.

THE ROOT

The root constitutes the underground part of the plant axis, specialized as an absorbing and anchoring organ. It occurs in the sporophytes of the vascular plants. among vascular plants only Psilotales lack such an organ. The sporophytes of these Primitive tracheophytes are attached to the ground by means of rhizomes bearing hair like absorbing structures, the rhizoids, (Eames, 1936).

Much variability exists in the shape and structure of roots; there are storage roots, succulent roots, aerial roots, pneumatophore, climbing roots, prop roots or whether they contain symbiotic fungi (Mycorrhiza) Plants growing in dry soils usually have better developed root systems.

On the basis of origin there are two types of roots.

(1) Primary roots

(2) Adventitious roots

Primary roots develop from the apex of the embryo that is destined from its origin; to give rise to a root, and from the pericycle of relatively mature

parts of roots, while adventitious roots develop from other tissues of mature roots or from other parts of the plant body such as stems and leaves.

Roots vary widely in morphology (Weaver, 1926) and exhibit structural and developmental differences correlated with more or less pronounced physological specializations. (Guttenberg, 1940). Most dicots and gymnosperms possess a root system based on the tap root and its branches. The tap root produces the lateral or branch roots in an acropetal sequence, i.e. with the youngest lateral located nearest the apical meristem, the oldest nearest the base.

The root systems of monocots are commonly composed of stem-borne, adventitious roots (Guttenberg, 1940; Tomlinson, 1961). The roots are devoid of secondary growth and are relatively homogeneous in size and form.

ARRANGEMENT OF THE PRIMARY TISSUES IN THE ROOT

At a certain distance from the apical initials of the root the following zones can be distinguished : root cap, epidermis, root cortex, and vascular or central cylinder.

Root Cap

The root cap is situated at the tip of roots it protects the root promeristem and aids the penetration of the growing root into the soil. The root cap consists of living parenchyma cells which often contain starch. These cells may have no special arrangement or they may be arranged in radiating rows which originate from the initials. In many plants the central cells of the root cap form a more distinct and constant structure which is termed the columella.

Root cap cell secrete a polysaccharide slime. It was found that the process of secretion is accompanied by hypertrophy of the dictyosome cisternae forming large vescicles. The secretion moves outwards through the wall. (Northcote & Pickett Heaps, 1966; Morre et al., 1967; Juniper & Pask, 1973; Paul & Jones, 1976).

Root cap controls the geotropic growth of the root. The root cap develops continuously the outer most cells die, become separated from one another, and disintegrate, and they are replaced by new cells which are produced by the initials.

Epidermis

The epidermal cells of roots are thin walled and

usually devoid of cuticle, although sometimes the outermost cell walls, including those of roots hairs, undergo cutinization (Guttenberg 1940; Scott et al, 1963). The root epidermis is typically uniseriate. A well known example of a multiseriate epidermis is the velamen of air roots of tropical Orchidaceae and epiphytic Araceae and of some terrestrial monocolytedons (Gessner, 1956; Mulay and Deshpande, 1959).

The most characteristic feature of the root epidermis is the production of root hairs which are organs well adapted to the efficient uptake of water and salts. The region of root hairs is usually restricted to one or a few centimetres from the root apex. Root hairs are absent close to the apical meristem and they usually die and dry out on the more mature portions of the root. Calcium is one of the factors controlling the normal development of root-hairs (Cormack et al 1963). In some plants the root hairs remain on the root for a long time. Long lived root hairs have been found in certain species of Compositae and in some plants of other families (Cormack, 1949; Scott et al 1963) but it is doubtful whether these root hairs take part in the uptake of water from the soil.

Epidermal cells of young roots and root hairs of

some genera secrete fibrilar mucilage forming a mucilaginous layer (Greaves and Darbyshire, 972; Leppard 1974). In plants grown on soil this layer was found to be colonized by bacteria (Foster and Rovira, 1976). In Sorghum in addition to fibrilar mucilage, drops of mucilaginous character are secreted near the tip of the root hairs (Werker and Kislev, 1978).

Root Cortex

In most of the dicots and gymnosperms the cortex of root consists mainly of parenchyma cells. The root cortex is usually wider than the stem cortex and therefore it plays a larger role in storage. The inner most layer of the cortex constitutes the endodermis. In certain plants such as Smilax, Iris, Citrus, (Cossmann, 1940) and Phoenix there is a special layer below epidermis termed as exodermis.

The parenchyma cells of the root cortex usually lack chlorophyll. Chloroplast are found in the roots only of certain water plants and in aerial roots of many epiphytes. Secretory cells, resin ducts and laticifers are found in the root cortex of different plants. If sclerenchyma is developed it is usually in the form of a cylinder within the epidermis within the

exodermis or adjacent to the endodermis.

In many gymnosperms & certain dicots such as plants belonging to the Cruciferae, Rosaceae reticulate or annular wall thickenings can be found in the cells outside the endodermis. Collenchyma is sometimes also found in the root cortex (Guttenberg, 1940).

Exodermis

In many plants the wall of the outer subepidermal layers of the cortex become suberized. In this way the exodermis, a protective tissue, is formed. (Guttenberg, 1943). The exodermis is similar in structure and cytochemical characteristics to the endodermis (van Fleet, 1950).

The thickness of the exodermis varies from a single cell layer to many layers. The exodermis may sometimes be accompanied, on its inner side, by sclerenchyma as, for example, in the root of Ananas (Krauss, 1949) of some grasses and Cypraceae. In Phoenix the exodermis is fibrous (Tomlinson, 1961).

Endodermis

The endodermis consists of uniseriate cylinder of cells and develops apart from a few exceptions, in all vascular plants. This layer of cells represents the

inner boundary of the root cortex. In that part of root where the primary vascular system is starting to mature, casparian strips appear in the radial and cross walls of the endodermal cells. These strips are not merely wall thickenings but integral parts of the primary wall and the middle lamella in which suberin and lignin are deposited (van Fleet, 1942a).

In the electron microscope the casparian strip appears as a slightly thickened homogeneous electron opaque wall region. If plasmolysis is brought about in endodermal cells the protoplast withdraws from the tangential walls but remains attached to the casparian strips. (Guttenberg, 1943). This type of plasmolysis has been termed band plasmolysis. The casparian strips prevent the inward flow of water and nutrients through the apoplast (Clarkson and Robards, 1975).

In many of the angiosperms, pteridophytes and some gymnosperms, the endodermis remains in the primary form and is shed together with the cortex with the development of secondary thickening and periderm. In other angiosperms in which there is no secondary thickening an almost continuous lamella of suberin develops on the inner side of entire primary wall

including the casparian strip. The production of suberin lamellae on the endodermal cell walls results from the polymerization of unsaturated fatty compounds which is brought about by oxidases and peroxidases. The peroxidases are brought to the endodermal cells via the sieve elements. This has led van Fleet (1942b) to suggest that this is the reason why the greatest amount of suberin is laid down on the inner walls of the endodermal cells and why the passage cells, which lack suberin, appear mostly opposite the xylem, and not the phloem, strands.

Vascular Cylinder

The vascular cylinder occupies the central portion of the root. The primary vascular tissue is surrounded by a region of cells which is termed as pericycle. The pericycle generally consists of one or more layers of thin walled parenchyma cells. It is in direct contact with proto-phloem and proto-xylem. The primordia of the lateral roots in all spermatophytes and the phellogen and portions of vascular cambium in dicots develop from the pericycle. In monocots the phellogen usually develops in the outer parts of the cortex. In roots of many Gramineae and Cyperaceae the outermost elements of the protoxylem may develop in the regions of the

pericycle. In such cases the pericycle is not continuous. (Guttenberg, 1943).

In the primary body of root the pericycle is bordered directly on its inner surface by the phloem and xylem strands. The phloem strands are always separate and they are concentrated on the periphery of the vascular cylinder. The xylem strands may be in seperate units on the periphery of vascular cylinder or they may extend into the centre and then it appears star-shaped in cross section. This structure led many workers to regard the vascular cylinder of the root as being a protostele.

The number of protoxylem groups in the root, i.e, whether one, two, three etc, is expressed by the terms monarch, diarch, triarch respectively, and the root in which there are many protoxylem groups is said to be polyarch. Monarch roots are found in small roots of Araucariacae (Guttenberg, 1941). Diarch roots are found in Lycopersicon Nicotiana, Beta, Raphanus and Linum. In Pisum the root is triarch, while in Vicia, Ranunculus and Gossypium it is tetrarch. Polyarch arrangement is characteristic of the adventitious roots of monocotyledons. Variations in these features may be

found even within the same plant. For example in one plant of Libocedrus decurrens di-, tri-, tetra-, penta-, and hexarch roots have been found. (Wilcox, 1962). Similar variations have been observed in certain dicotyledonous species (Jost, 1932; Torrey, 1957).

In most plants there are inter connections between different tracheary elements which appear separate in cross section of the root. The primary phloem of the roots of most plants does not contain fibres but in certain plants such as those of Papilionaceae, Malvaceae & Annonaceae (Guttenberg, 1943) fibres are found in the primary phloem.

Some roots are polystelic. In corss-section of such roots a number of vascular cylinders each of which is surrounded by an endodermis. Examples of such roots are the root tubers of some species of Orchis (Arber, 1925) and members of the Palmae (Tomlinson, 1961).

CAMBIUM IN ROOTS

There is great variation in the secondary growth in different roots. The tap root and main lateral roots of gymnosperms and woody dicotyledons usually have secondary thickenings, but the smallest branches do not.

In the roots of some herbaceous dicotyledons secondary thickening may be completely absent (eg. Ranunculus) or may be well developed (eg. Medicago).

Roots of most monocotyledons are devoid of secondary thickening, but in some it occurs (eg., Dracaena).

In the roots of gymnosperms and dicotyledons that do exhibit secondary thickening the cambium first appears on the inner side of the phloem. After these cambial cells have produced a few secondary elements the pericylce cells on the outer sides of protoxylem groups begin to divide, and the inner cells resulting from these divisions form cambial cells. At first the cambium has an undulating shape but as the development of the secondary xylem on the inner side of the phloem strand precedes that of the secondary xylem external to the protoxylem groups, the cambium soon becomes circular.

DEVELOPMENT OF LATERAL ROOTS

Lateral root primordia seem to occur in many plants in a more or less regular sequence (Riopel 1966; Mallory et al, 1970). It is found that the smaller the number of protoxylem poles (potential sites of lateral root formation) the greater the degree of regularity in

the arrangement of the lateral roots. The spacing of lateral root primordia in horizontal plane is determined by their relationship to the developing vascular cambium system.

In longitudinal plane, several factors seem to control the spacing of lateral root primordia. A definite relationship exists between the position of the protoxylem groups and phloem strands and the position of the initiation of lateral roots.

In angiosperms the primordia of the lateral roots are formed by the periclinal and anticlinal divisions of a group of pericycle cells. In many plants eg. Daucus Carota and Zea mays the endodermis of the parent root takes part in the formation of the primordium of lateral roots (Esau, 1940; Bell and McCully, 1970). In some plants the derivatives of the endodermis,. together with those of other cortical layers, form a root cap like structure called pocket (Guttenberg, 1960). The pocket is well developed in water plants in which the root-cap may be lacking e.g. Eichhornia, Hydrocharis and Lemna.

STRUCTURE OF STORAGE ROOTS

In all primary roots reserve substances (mostly

starch) are stored in the cortex, which in most plants is relatively thick. In ordinary roots with secondary reserve substances are stored as in stem i.e. in the parenchyma and sclerenchyma tissues of the secondary phloem.

In some plants certain parts of root system develops into thick fleshy organs which functions especially as storage organs. In many plants the tap root and hypocotyle undergo such modification. The origin of the storage tissue may differ in carrot, for example (Esau, 1940) the hypocotyl and tap root become thickened and with the development of periderm, the narrow cortex is shed. The organ become fleshy as a result of excessive development of parenchyma in secondary xylem and especially in the secondary phloem.

In radish the fleshiness of the root and hypocotyl is due to excessive development of parenchyma in the secondary xylem, as well as secondary parenchyma produced by additional cambia (Hayward, 1938).

CONTRACTILE ROOTS

The renewal buds of certain plants occupy a definite position within the soil or on its surface.

This position is obtained by the pull of special roots which have been termed contractile roots (Rimbach, 1895, 1899, 1929, 1932, Arber, 1925; Bottum 1941; Davey, 1946; Dittmer, 1948; Galil, 1958, 1968) Such roots exist in many herbaceous dicotyledons e.g. Taraxacum, Medicago sativa, Daucus, Oxalis, sugar beet and in many bulbous and cormous monocotyledons e.g. Hypoxis setosa, Gladiolus segetum Colchicum steveni.

Contractile roots or parts of roots are distinguishable from normal roots by their outer, wrinkled appearance. Contraction of roots is a result of cellular growth and collapse. According to Arber (1925), who studied Hypoxis setosa, only the outer cortex is wrinkled whereas the central cylinder and inner cortex are unaffected. Rimbach (1899) and later investigators explained the shortening of the inner core as being due to the change in the form of inner cortical cells. These cells, according to them, increase in radial and tangential diameter and decrease in length.

AERIAL ROOTS

Many tropical plants eg. some Ficus species, Rhizophora, Orchidaceae produces roots from the stem or branches which remain free in the air. If they grow

downwards into the soil they serve as prop roots. If they attach to solid objects, they represent climbing or adhesive roots. These aerial roots exhibit special adaptive anatomical characters. The cortical cells frequently contain chloroplasts, and in some cases (eg. Toemophyllum) the roots take over the main photosynthetic activity.

It was suggested that absorption of water is accomplished with the aid of a specialized multiseriate epidermis, the velamen. The velamen is a sheath of compactly arranged dead cells, the walls of which are strengthened by band like or reticulate-thickenings and which contain many primary pit-fields. When the air is dry, these cells are filled with air, but when rain falls they become filled with water. The exodermis situated at the inner edge of the velamen is interrupted by thin walled passage cells through which the absorbed water may be transferred. Special structures termed as pneumatodes are present in the velamen. There function is to enable gas exchange during the periods when root is saturated with moisture. The principal role of the velamen are mechanical protection and prevention of excess loss of water from the cortex. (Dycus & Knudson 1957; Gill & Tomlinson 1975).

MATERIAL & METHOD

SELECTION OF THE MATERIAL

The plants are selected on the basis of their medicinal value especially their, root, stem, xylem, phloem and leaf. For selecting the material the plants should be healthy, green without any patches or diseases. These are as follows.

(1) **Anagalis** (Primulaceae)
(Jonk mari)

(2) **Bacopa monniera** (Scrophulariaceae)
(Shwet chamni)

(3) **Calendula officinalis** (Compositae)
(Marigold)

(4) **Centella asiatica** (Apiaceae)
(Brahmi)

(5) **Echinops echinatus** (Compositae)
(Gopa-Gokhru)

(6) **Sphaeranthus indicus** (Compositae)
(Gul-e-mundi)

COLLECTION OF THE MATERIAL :

For collection of the material healthy plants should be selected. In case of bark, if present, samples of one inch square size will be taken having cambium, will be collected from the main trunk. The collection will be made from 3 - 5 adult plants to study the material and its components.

The collection of leaf, will comprise both types of leaves, fairly old and young to study the morphological and anatomical features. The material will be fixed on the spot.

FIXING AND PRESERVATION OF THE MATERIAL :

After taking out the samples they are immediately fixed on the spot, either with F.A.A. or craf III. Later they are aspirated for the free access of the fixative into the deep lying tissue after reaching the laboratory. The samples will be allowed to remain in the fixative for atleast 6 days, while the young leaves will be fixed in Carnoy's solution for one hour. After a week, they are transferred to 70% ethanol or alco glycerol for softening and preserving.

(a) F.A.A. (Formalin Aceto Acetic Acid)

It is a mixture of glacial acetic acid, formalin, and alcohol in following ratio -

Formalin	-	5 cc.
Glacial CH_3COOH	-	5 cc.
Ethyl Alcohol 95%	-	90 cc.

(b) Craf III

It is a mixture of chromic acid, acetic acid, formaldehyde and water in following proportion.

1%	Chromic acid	-	30 cc.
10%	Acetic acid (not glacial)	-	20 cc.
	Formaldehyde 37 - 40% aqueous	-	10 cc.
	Water	-	40 cc.

(c) **Carnoy's Fluid**

It is a mixture of anhydrous ethyl alcohol, chloroform and glacial acetic acid in the following proportion -

Anhydrous ethyl alcohol	-	60 cc.
Chloroform	-	30 cc.
Glacial acetic acid	-	10 cc.

(d) **Alco Glycerol**

This mixture comprises of the following proportion of glycerine and alcohol.

50%	Glycerine	-	50 cc.
50%	Ethanol	-	50 cc.

SECTIONING OF THE MATERFIAL

After getting preserved the material will be sectioned.

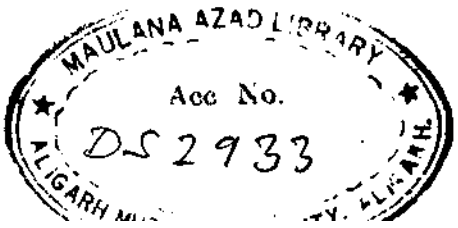
For sectioning the samples will be washed thoroughly in running water. the sectioning will be done in transverse and longitudnal planes on Reichert's

sliding microtome at a thickness of 10 - 12 um. For this first of all material will be cut into small slices and then placed in the clamp on hand adjustment, in such a way that it should almost touch the underside of knife. The knife will be oriented at a wide oblique angle in order so that as much of the length of the blade is used for cutting purposes. The knife should be kept sharpened and free from nicks. Before sectioning the material should be moistened with water by means of camel brush, if preserved in 50% alcohol. The sections will be cut in first stroke, should be moved towards the back of knife with the help of brush and should be left for few minutes to avoid curling. In the last, the best sections will be chosen and picked for staining and mounting.

For soft material sections will be cut on rotary microtome and slides will be processed for studies following the method given below :

HISTOLOGICAL STUDIES

The parts of plant such as root, Bark etc. will be collected and gently washed to remove all soil particles. The material will be cut into small pieces and processed.



FIXATION

The material collected will be washed and fixed on F.A.A. which will be prepared as under :

Ethanol	50%	-	90 ml
Formalin	37%	-	5 ml
Glacial Acetic Acid		-	5 ml

The duration of fixation depends upon thickness of material from 24 hours to several days.

DEHYDRATION

The dehydration schedule will be carried out according to Johansan (1940) Tertiary Butyl Alcohol (T.B.A.) will be used for dehydration (Table I).

INFILTRATION

In this step alcohol in the tissues will be replaced by paraffin so that tissue is saturated with pure solution of paraffin.

After following T.B.A. dehydration schedule 100% T.B.A. in step 8 will be replaced by 1 : 1 mixture of 100% T.B.A. and paraffin oil. The tissues will be allowed to remain in this solution for 1 hour. Shortly after that, another container will be filled with 3/4 of its volume by paraffin oil and material kept in this.

TABLE - I

Step	%Alcohol	Time	Quantity needed for solution			
			Distilled Water	95% Ethanol	100% Ethanol	100 TBA
1.	50	2 hr.or more	50	40	0	10
2.	70	over night	30	50	0	20
3.	85	1-2 hr.	15	50	0	35
4.	95	1-2 hr.	0	45	0	55
5.	100	1-3 hr.	0	0	25	75
6.	100	1-3 hr.	0	0	0	100
7.	100	1-3 hr.	0	0	0	100
8.	100	over night	0	0	0	100

It is important to keep the Tertiary Alcohol changes in warm place as the chemical solidifies at 25.5°C.

The material from T.B.A. paraffin oil mixture will then be placed on top of the solidified paraffin oil solution. This container will be placed uncovered in an oven set at slightly above melting point of paraffin. After 1 - 3 hours the T.B.A. paraffin oil mixture will be poured off and replaced with pure melted paraffin wax and kept in oven for about 3 hours. The step will be repeated at least once more.

EMBEDDING

The material will be placed in metal base mold or folded paper. Molds will be first coated with thin layer of glycerine and thin liquid paraffin will be poured. Roots will be placed into mold with heated forceps and additional melted paraffin will be addeed to fill the mold. Once the paraffin begin to solidify at the top of mold, the mold be plunged into the ice water and left there until solidification. After hardening it will be cut into smaller blocks and trimmed. Then the blocks will be mounted on block holders.

SECTIONING

Transverse and longitudinal sections of 8 - 12 um thickness of the material will be cut serially will the help of rotary microtome. The paraffin ribbon thus

obtained will mounted on a clear glass slide with an amount of albumin and glycerine dissolved in water. The slide will be kept over night in an incubator at 40°C to allow water to evaporate. These slides will then be kept at 60°C for an hour to melt the paraffin.

STAINING

The process of staining will remove all paraffin from the sections increase the contrast in the tissues. Staining will be done with safranin and fast green comibation (Table - 2) (Sass, 1951). Then slides will be removed from the xylene and laid on flat absorbant surface. The mounting medium will be applied to surface of the slide before evaporation of xylene and cover slip will be lowered gradually over the slide.

Finalized slides will be left to dry for at least 24 hours at room temp. The medium will harden better if the slides are hold on 60°C warming tray over night. The slides will examined and observation taken.

TABLE - II

SAFRANIN AND FAST GREEN STAIN

Step	Solution	Time
1.	Xylene	5 min.
2.	Absolute ethanol	5 min.
3.	95% ethanol	5 min.
4.	70% ethanol	5 min.
5.	50% ethanol	5 min.
6.	30% ethanol	5 min.
7.	1% aqueous safranin	1 - 12 hr.
8.	Rinse in tap water	
9.	30% ethanol	3 min.
10.	50% ethanol	3 min.
11.	70% ethanol	3 min.
12.	95% ethanol	3 min.
13.	0.1% fast green in 5% ethanol	5 - 30 sec.
14.	Absolute ethanol	15 seconds
15.	Absolute ethanol	3 min.
16.	Xylene - absolute ethanol 1:1	5 min.
17.	Xylene	5 min.
18.	Xylene	5 min. or longer

MACERATION

To study the morphological details of the elements pieces of them will be macerated using suitable reagents. For maceration of sieve elements sclereids, phloem parenchyma etc. the samples will be cut into thin tangential slices of 0.5 - 1 mm thickness . These slices will be treated with 5% NaOH solution at 45 - 50°C. The treatment will be continued till the cells of slices become sufficiently loose to allow the separation of individual elements, on a slide with a slight pressure when applied over the coverslip after mounting in 5% glycerine (Ghouse et al 1974) when the desired stage is found they will be washed and stained 1% aqueous solution of Astra blue or lacmoid. In case of phloem fibres and sclereids the material will be treated with safranin. Lacmoid was preferred in winter because it made clear the closure of sieve pores by callose.

STAINING AND DEHYDRATION

STAINS

The sections will be stained with a number of stains alone or in combination depending on the nature of study.

(A) For the study of root, and stem the following stains will be used :

(1) Heidenhain's Haematoxylin and Bismark Brown

(2) Heidenhain's Haematoxylin & Safranin.

(3) Safranin & Fast Green.

(4) Foster's Tannic Acid - Ferri Chloride - Lacmoid.

(5) Flemming's - Triple Stain.

(B) <u>For the study of Macerated Elements</u> :

For macerated elements, Astra blue will be used for sieve tube elements & safranin for fibres.

In all the cases the material will be dehydrated in ethyl alcohol series & passed through clove oil, xylene and finally will be mounted in Canada Balsam.

(C) <u>For the study of Leaf</u> :

<u>Epidermal Studies</u>

<u>Preparation of Epidermal Peelings</u> :

For the preparation of epidermal peels any one of the following methods will be tried :

(A) <u>Ghouse & Yunus (1972)</u>

The leaf will be cut into small pieces and boiled with 40 - 60% HNO_3 (Nitric acid) for 2 - 3 min. in a test tube. Simultaneous shaking of the tube makes easy removal. Then the peelings are thoroughly washed with water and later on neutralized by 1% solution of alkali

(NaOH or KOH). It is further washed to remove the trace of alkali.

(B) Ram & Nayyar (1974)

The leaf will be cut into desired size with one edge left untrimmed from the margin. The cut pieces of leaf are boiled in 5 - 10% solution of cupric sulphate for 1 - 2 minutes duration. Later on 4 unit of Conc. Hcl (Hydrochloric acid) is added and again boiled for 1- 2 minutes resulting in the break down of internal tissues. With the aid of fine brushes the mesophyll is removed and like opened book the epidermis of two surfaces is ready for staining after thorough washing with water. The epidermal peelings will be stained with Heidenhain's Haematoxylin-safranin/Bismark brown for older leaves.

The peelings from young ones will follow the Paliwal's procedure. The peels obtained will be analysed taking into account the stomatal size, stomatal number and the statistical analysis of stomata.

MEASUREMENT OF STOMATAL SIZE

The size (Length & width) of stomata will be measured with micrometer occular scale under light microscope. The least count at a particular magnification

will be calculated by coinciding the divisions of micrometer occular to micrometer scale. The multiplication of the least count to the size measured by micrometer occular scale would give the actual size in milimicron.

STOMATAL NUMBER

The average number of stomata per sq. mm. of epidermis is termed as the stomatal number. In recording results the range as well as the average value were recorded for each surface of the leaf and the ratio between the two surfaces.

STATISTICAL ANALYSIS OF STOMATA

The stomatal index in about ten samples of each species will be calculated following the method described by salisbury (1927) and for each sample about 100 readings will be taken at random from both the surfaces of the leaf.

$$Is = \frac{S}{E + S} \times 100$$

Where, Is = Stomatal index

S = Number of Stomata at a particular magnification.

E = Number of epidermal cells at the same magnification.

(1) HEIDENHAIN'S IRON HAEMATOXYLIN & BISMARK BROWN (JOHANSAN, 1940) :

The stains which will be used are prepared by the process given below.

IRON ALUM (Ferric Ammonium Sulphate)

To be used as a mordant or a fixer to the stain. 2% solution of Iron Alum is prepared in distilled water.

HEIDENHAIN'S HAEMATOXYLIN

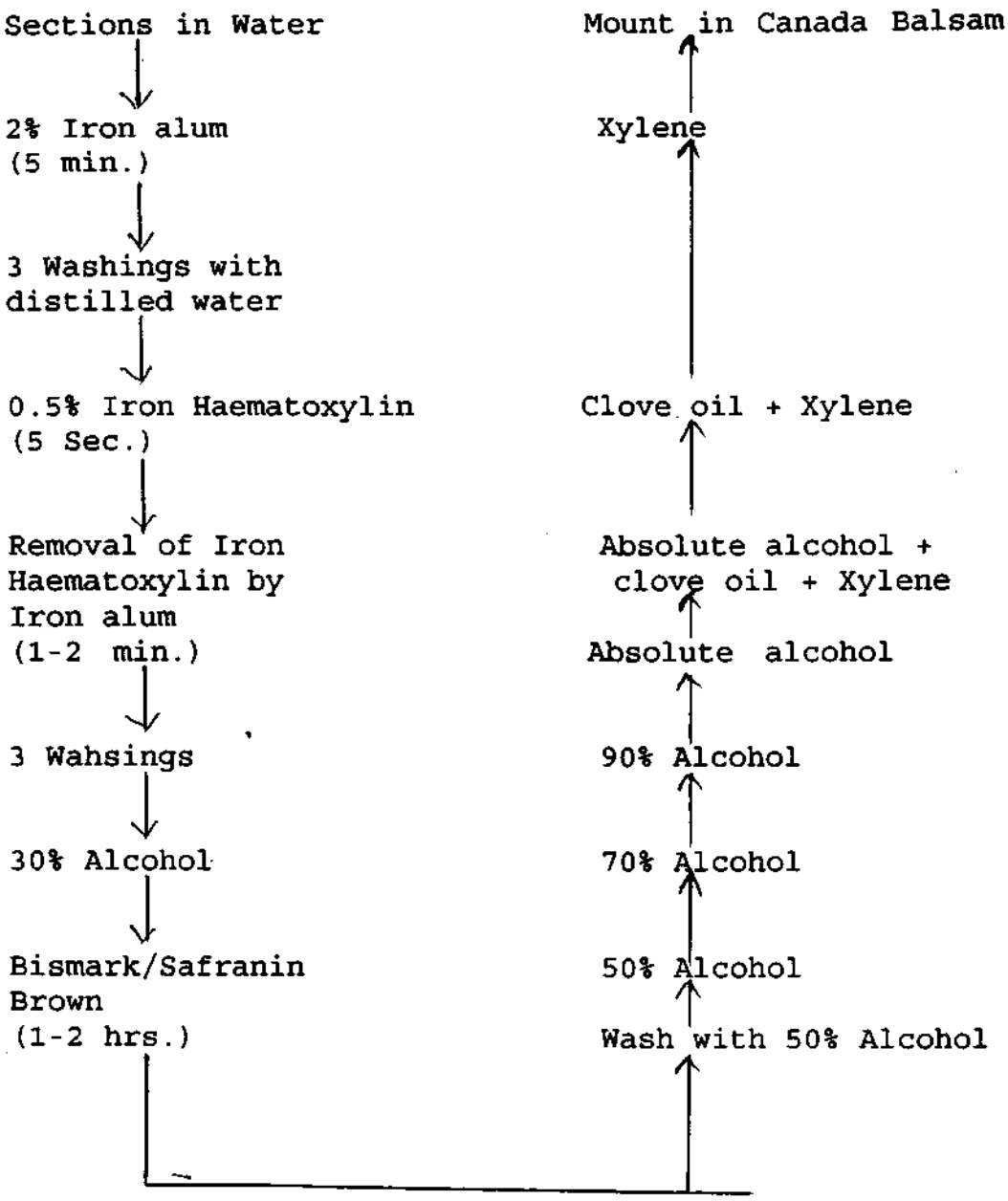
0.5% solution of the dye is prepared by dissolving the stain in 100 cc. of distilled water. This stain has little or no affinity for tissues unless Iron (always in ferric form) or aluminium is present in latter. This stains middle lamella and nuclear material.

BISMARK BROWN

Solution is prepared by dissolving 1gm of the dye in 100 cc. of 70% alcohol. It stains the lignified walls of fibres and sclereids brown.

STAINING SCHEDULE

1. HEIDENHAINS HAEMATOXYLIN & BISMARK BROWN :



(2) HEIDENHAIN'S HAEMATOXYLIN AND SAFRANIN

Preparation of Safranin :

It is made by dissolving 2.25 gms. of dye in 225 cc. of 95% Alcohol and diluted with equal amount of water when needed. It stains cutin, chromatin, lignin and in some chloroplasts.

STAINING SCHEDULE :

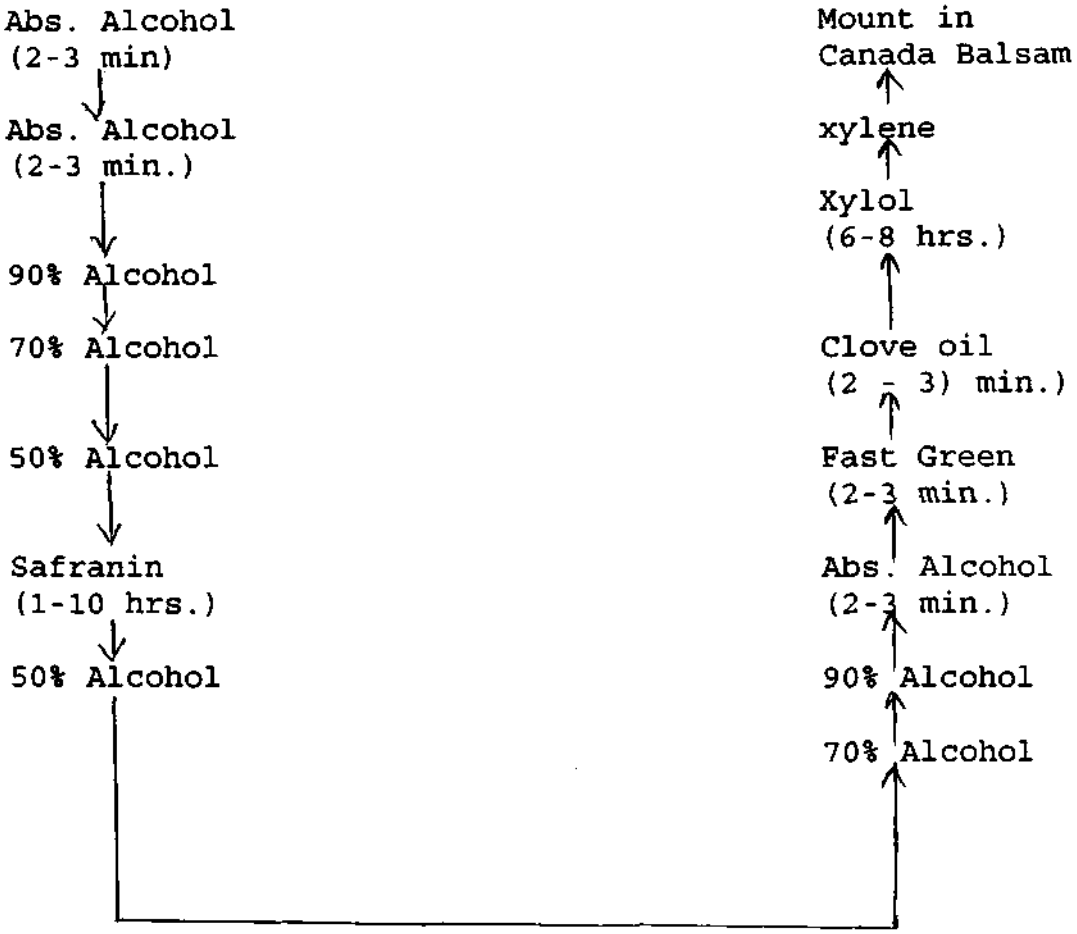
Staining procedure is same as that of Haematoxylin and Bismark Brown, except that Bismark Brown is replaced by safranin. Both of them stain lignified wall brown and red respectively. (Johansan, 1940).

(3) SAFRANIN AND FAST GREEN

It is very common stain, In this staining schedule, first of all sections are treated with aqueous safranin for 2 - 12 hrs, and then dehydrated. After reaching upto 90% alcohol, sections are treated with fast green in clove oil medium for 3 - 5 seconds. The excess of fast green is removed in clove oil. The sections are cleaned in xylene and finally mounted in Canada Balsam.

STAINING SCHEDULE

3. SAFRANIN & FAST GREEN



(4) Foster's Tannic Acid - Ferric Chloride _ Lacmoid

(Chedle et al., 1953)

Preparation of Tannic Acid :

1 gm tannic acid dissolved in 100 cc. of water.

Lacmoid or Resorcin Blue :

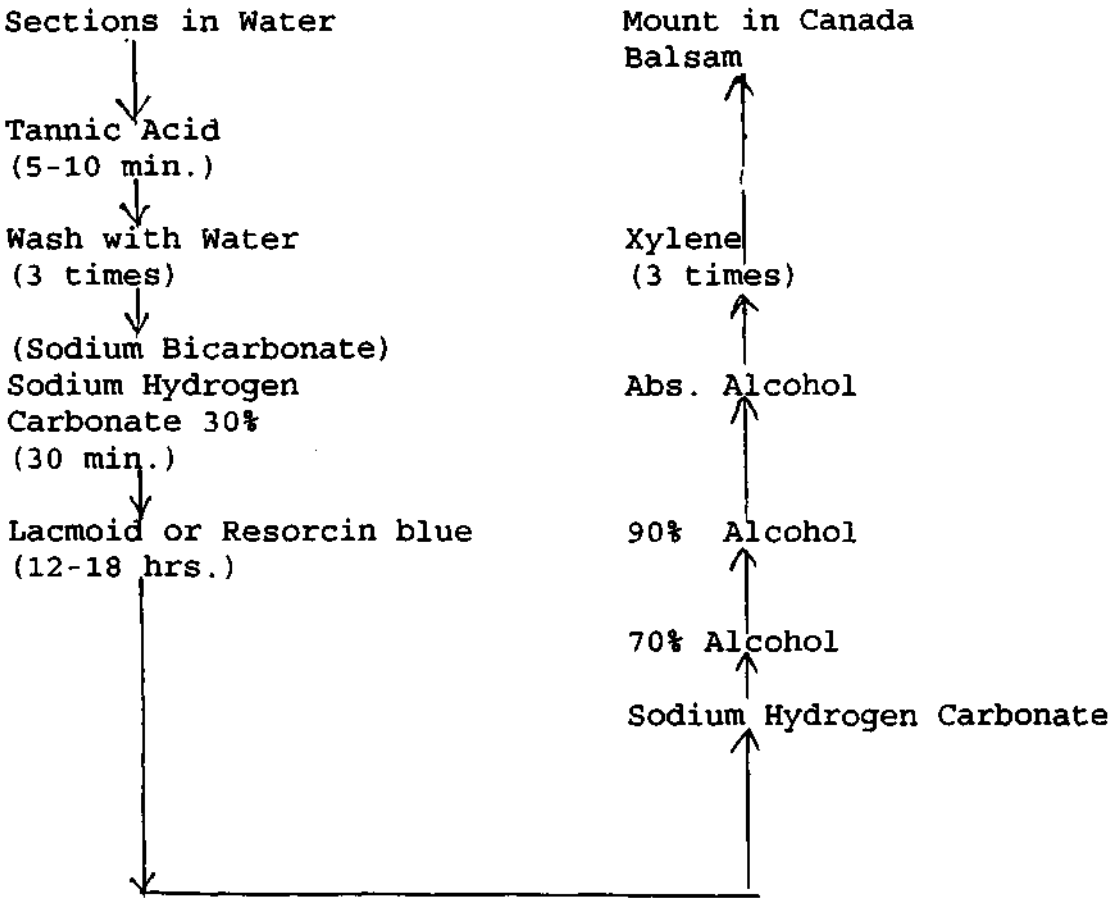
It is used for callose detection and stains blue 2% solution is prepared by dissolving 2gm Lacmoid in 100 cc. of 50% Ethanol.

Sodium Hydrogen Carbonate :

1% solution is prepared by dissolving 1 gm of sodium hydrogen carbonate in 100 cc. of absolute alcohol. This is used as mordant for Lacmoid.

STAINING SCHEDULE

(4) Foster's Tannic Acid - Ferric Chloride _ Lacmoid



(5) FLEMMING'S TRIPLE STAIN

(Johansan, 1940)

It is called triple stain because it involves three stains : Safranin-which stains lignified cell wall red; Orange G-stains cytoplams; Crystal voilet-stains cellulose wall voilet.

Preparation of Safranin :

Solution is made by dissolving 2.25 gms dye in 225 cc. of 90% alcohol and diluted with equal amount of water, when needed. It stains cutin, chromatin, lignin and in some cases chloroplasts.

Crystal Voilet :

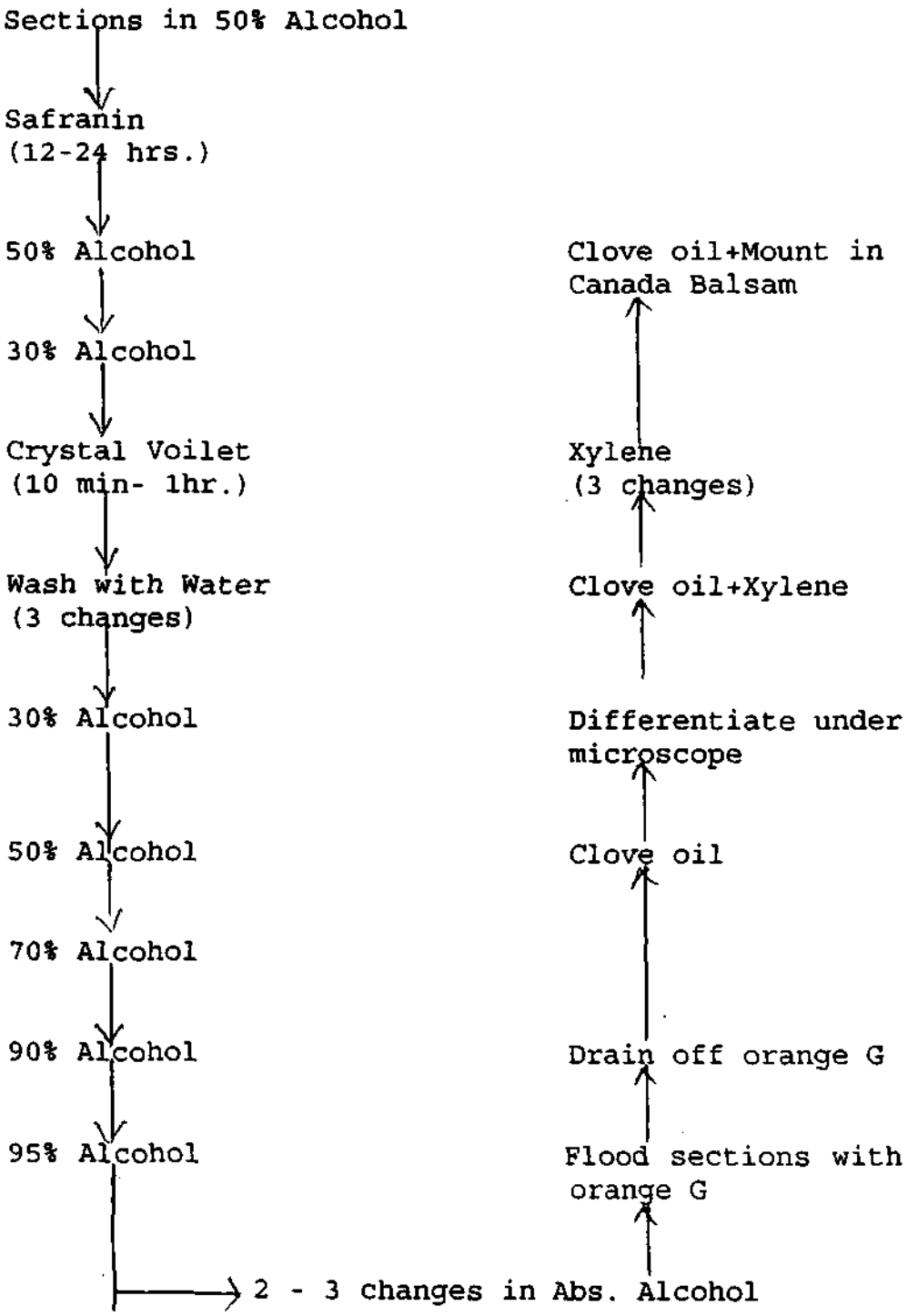
1% solution prepared in distilled water, it frequently stains cellulose wall voilet.

Orange G :

1% solution of the dye prepared in absolute alcohol is used. It is a useful cytoplasmic stain.

STAINING SCHEDULE

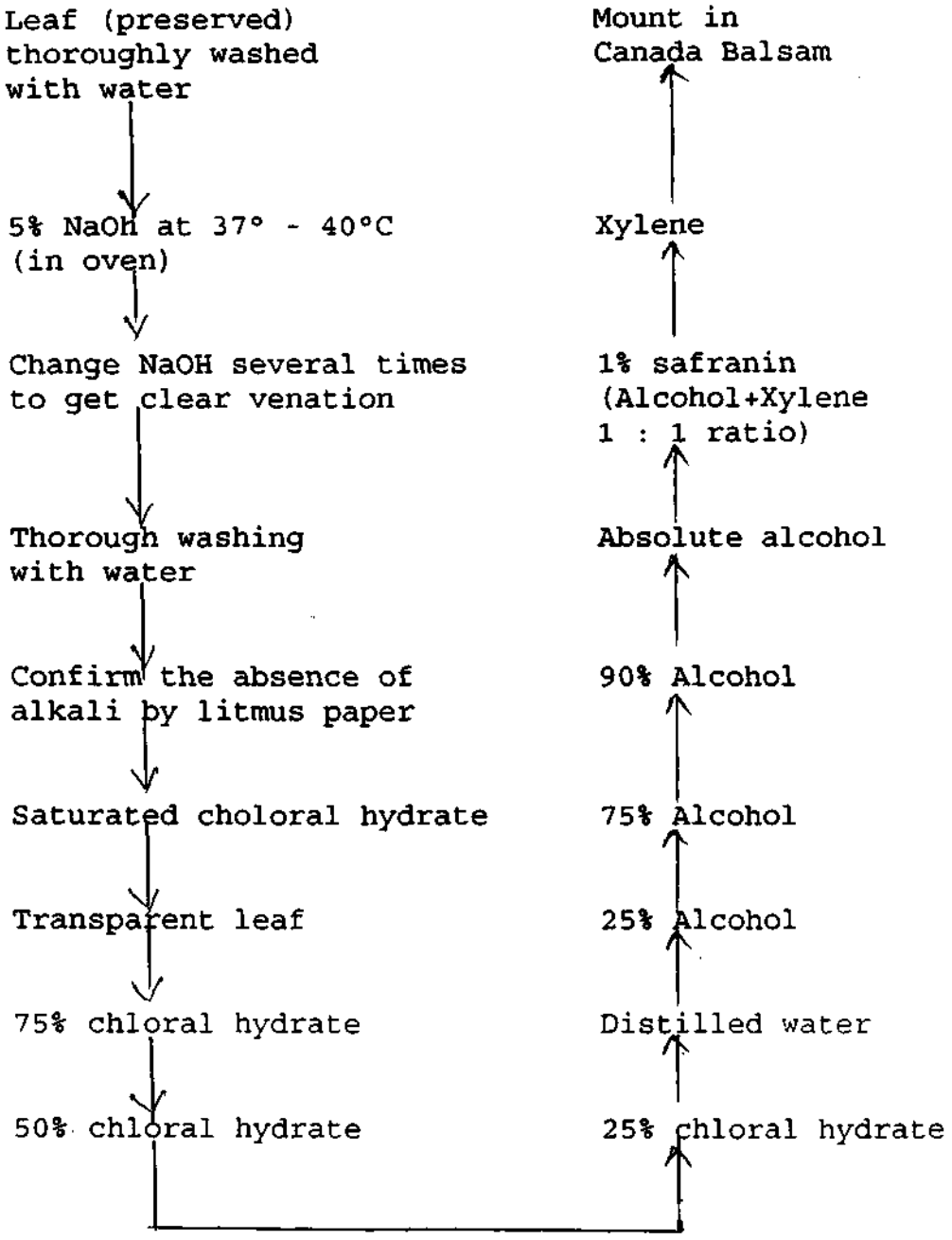
5. TRIPLE - STAIN



(6) PALIWAL AND KAKKAR'S TECHNIQUE

(After Paliwal and Kakkar, 1969)

STAINING SCHEDULE



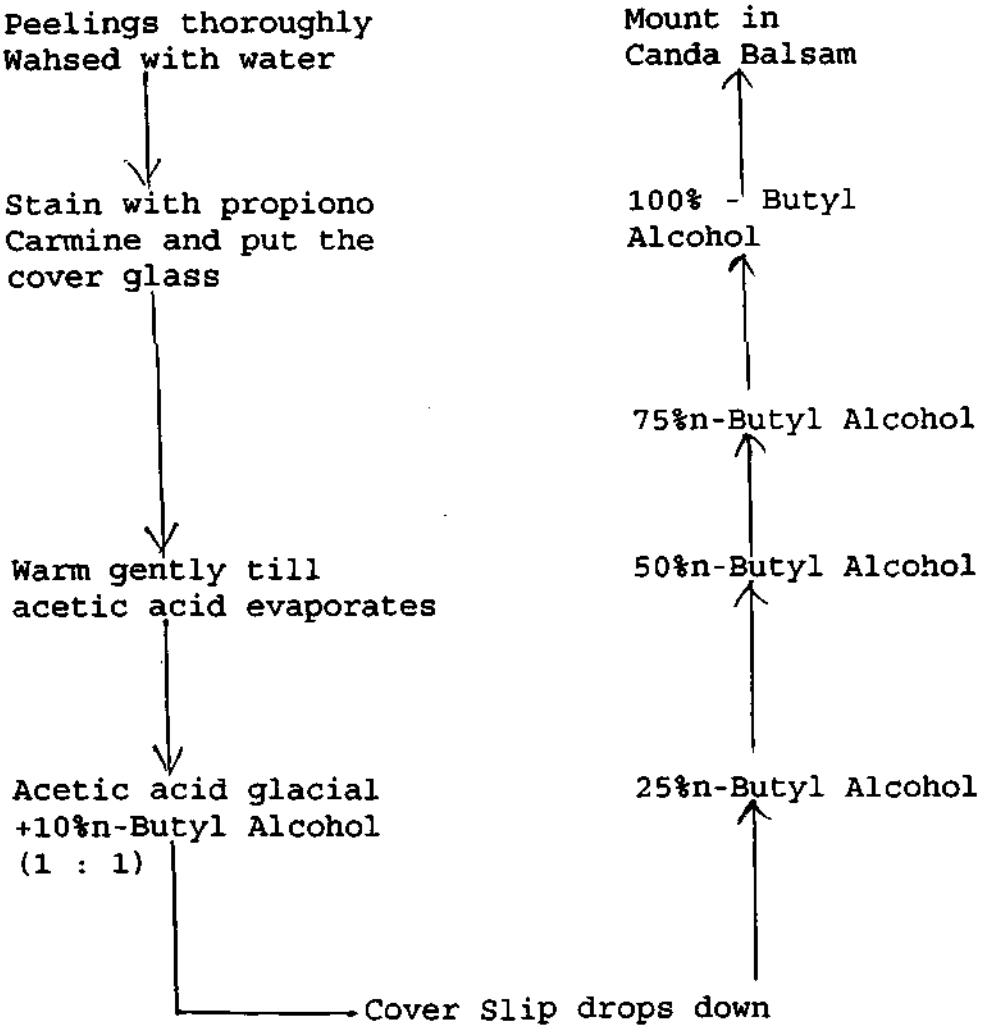
(7) PALIWAL'S TECHNIQUE

(After Paliwal, 1967)

Preparation of Propiono carmine

1 gm carmine powder is boiled with 100 cc. of 45% propionic acid for 1-2 min. Until there is sudden change to darker colour, the stain is cooled and filtered.

STAINING SCHEDULE



HISTO CHEMICAL TESTS

Following tests will be conducted on the selected samples in order to detect the colour response of tissue to different chemical reagents. For this only fresh sections which should be 15 - 30 mm thick will be used.

TEST FOR LIGNIN:

There are 2 tests:

(1) Fresh sections will be treated with phloroglucinol solution, followed by Hcl & then they will be examined under microscope. Appearance of red colour on lignified walls will show the presence of lignin.

(2) Fresh sections treated with aniline sulphate solution & then with H_2So_4 will give yellow colour to lignified cell walls.

Preparation of Phloroglucinol:

1% of phloroglycinol is dissolved in 50 ml of 95% alcohol.

TEST FOR SUBERIN:

There are 2 tests:

(1) Sections treated with Sudan (III) solution. This will produce brownish to red rose colour in the

suberized walls.

(2) Sections treated with 10% aqueous solution of KoH & then with H_2So_4. Suberized walls will stain yellow.

COMBINE TEST FOR CELLULOSE & LIGNIN

Fresh sections will be treated with chloro zinc iodine solution which will stain cellulose wall blue or voilet while lignified wall will be yellow.

Preparation

25 gms of anhydrous zinc chloride and 8 gms of KI crystals are added to make a saturated solution.

TEST FOR CALLOSE

It is polymerized type of sugar present on the lateral wall of sieve plates. It is synthesized & variously degraded.

There are several tests for distinguishing callose.

(1) First Test:

Fresh sections will be put for 30 minutes in a freshly prepared & not too strong aqueous solution of aniline blue & then will be tranferred on the slide. A small drop of Levulose syrup will be added (10 gm levulose + 80 ml of distilled water)

warm the slide and cover with cover slip. Callose will stain blue after the evaporation of syrup.

(2) Second Test:

In this test Rosalic acid will be used in place of Aniline blue 1% solution of Rosalic acid in 4% aqueous $Na_2(Co_3)$. Red colour will import on callose.

(3) Third Test:

Sections will be placed in a drop of 1:25 cc solution of Resorcin blue. Sections will be transferred on the slide & mounted in a drop of glycerineor Levulose syrup for examination.

TEST FOR ALKALOIDS:

First Test:

Sections treated with wagner's reagent will turn yellow indicating the presence of alkaloids.

Preparation of Wagner's Reagent:

1.27 gm of Iodine & 2 gms of KI, dissolve in 5 ml. of H_2O & then dilute the solution to 100 ml.

Second Test: Sections treated with Dragendroff's reagent gives brown colour confirming the presence of alkaloids.

Preparation of Dragendroff's reagent: 8 gms of Bismuth nitrate+20 ml of HNO_3 in 7 gms of KI in 50 ml. of water.

TEST FOR FLAVINOIDS

They are found in very low concentration & are concentrated with Hcl.

Hcl added to ethanol extract will give red colour & will confirm the presence of flavinoids.

TEST FOR PHENOLS:

Leibermann's Reaction:

Sections are treated with the crystal solution of sodium nitrite after that sections will be cooled below $10^{o}C$ & 1 ml of conc. H_2SO_4 will be added. A deep green to blue colour will be formed which will turn red on adding excess of water. This deep red colour will become again green or blue if NaoH or KoH is added.

Secondary Test:

Add 2 drops of $FeCl_3$ after making the material aqueous or alcoholic in nature. Formation of green blue or voilet colour indicates the presence of phenols.

PRELIMINARY PHYTOCHEMICAL STUDIES

DETERMINATION OF PHYSICAL CONSTANTS

ASH VALUE

It is calculated by the methods given below:

1. Total Ash
2. Acid Insoluble Ash
3. Water Soluble Ash

(1) **Determination of Total Ash**:

5 gms of powdered drug will be put in a silica dish at a temperature not exceeding 450^{o}C in a mufle furnace until it becomes free from carbon, it will be then cooled and weighed. Before weighing the powder will be dried at 105^{o}C for removal of moisture. The percentage of ash will be calculated with reference to the weight of sample taken (Anonymous 1968)

(2) **Determination of Acid Insoluble Ash:**

In this the ash will be boiled for 5 min, with 25 ml of dil. Hcl, this insoluble matter will be collected on ashless filter paper washed with hot water & will be ignited at 450^{o}C like total ash. The percentage of Acid insoluble ash will be calculated with reference to the weight of the sample of powdered drugs. (Anonymous 1968)

(3) **Determination of Water Soluble Ash**

In this test ash will be boiled for 5 min, with 25 ml, of distilled H_2o This insoluble matter will be collected on ashless filter paper, washed with hot distilled water & ignited at 450^{o}C. The weight of insoluble matter will be subtracted from the weight of total ash. The difference in weight will give the weight of water soluble ash. The percentage will be calculated with reference to the weight of the sample of powdered drugs (Anonymous 1968)

EXTRACTION AND ESTIMATION OF CHEMICAL COMPONENTS

Extraction wil be made in soxhlet apparatus with different solvents i.e. petroleum ether, solvent ether chloroform, benzene, ethnol and water by taking known quantity of the drug. The extracts will be filtered & solvent will be evaporated, accurate weight of the extracts will be taken. The percentage will be calculated with reference to air dried drug.

QUALITATIVE ANALYSIS:

The drugs will be extracted with water and Ethanol & these extracts will be taken for various organic compounds which are given below :

TEST FOR FLAVINOIDS:

(a) Flavinoids will be tested by the formation of yellow colour with HCl & Magnesium respectively.

(b) Sodium hydroxide solution will be added to the test solution, yellow colour will indicate the presence of flavinoids.

TEST FOR GLYCOSIDES;

(a) Hydrolysis of the solution will be done with conc. H_2So_4 & after hydrolysis the presence of sugar will be determined with the help of fehling's

solution. A black red ppt. will indicate the presence of glycosides.

(b) Molisch's Test: In the aqueous solution naphthol will be mixed & conc. H_2So_4 will be poured gently. A purple colour will indicate the presence of glycosides.

TEST FOR STEROLS/TERPENES:

(a) Leibermann's Burchard Reaction: To 1 ml of extract, 2 ml of acetic anhydride solution will be added followed by 2 ml of H_2So_4. The colour change from red to blue will indicate the presence of Sterols/Terpenes.

(b) Hosses Reaction: In the test solution chloroform will be added followed by 2 ml. of conc. H_2So_4 poured from the side of the test tube. A red colour at the junction of the two layers will show the presence of sterols/Terpenes.

TET FOR ALKALOIDS

(a) In the test solution a drop of Mayer's reagent will be added. Appearance of white ppt will indicate the presence of Alkaloids.

(b) Ethanolic extracts acidified with HCl, & then 2 - 3 drops of Iodine will be added. Appearance of brown ppt will confirm the presence of Alkaloids.

TEST FOR TANNINS:

$FeCl_3$ solution will be added which will disappear on the addition of dil. H_2So_4 & will produce a yellow brown ppt indicating the presence of tannins.

TEST FOR RESINS:

0.1 gm. of Ethanolic extract will be heated and 10 ml. of acetic anhydride will be added to it will be then cooled down, after that one drop of H_2So_4 will be added. A purplish red colour changing to voilet, will indicate the presence of resin.

FLUORESCENE ANALYSIS

Fluorescence Analysis of the drug will be conducted by the method of Chase & Pratt (1949). The powder of the different drugs will be observed as such, under ultra voilet light. Subsequently, the samples will be treated with 1% NaoH in methanol & with saturated solutions of nitrocellulose in amyl acetate respectively & observed under ultra voilet light. Finally this powder will be mounted in nitrocellulose after treating it with NaoH followed by drying. The resultant colour will be observed under ultra voilet light.

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