I REVIEW OF LITERATURE

1. Historical perspectives :

The family Zygophyllaceae belongs to dicotyledons. It consist of 22 genera and about 100 species. Ecologically they can be classified as Xerophytes and hand halophytes distributed in tropical and sub-tropical parts of the world. The morphology and taxonomy has attracted the attention of many research workers.

With the advent of the discovery of Alkaloids, aromatic oils and active ingredients of the member of this family and their use in medicine have attracted attention of pharmaceutical industries. Today several members of this family have been reported to contain useful products such as volatile oils, alkaloids and glycocides as below :

Zygophyllum simplex Linn.
Zygophyllum coccineum Linn.
Fagonia cretica Linn.,
Tribulus terrestris Linn.,
Tribulus alatus Linn.

2. Tribulus :

Among 22 genera of Zygophyllaceae the genus Tribulus has about 25 species distributed throughout the warm regions of the globe. It is

found throughout India ascending to 3,385 metres (11,000') in Western Tibet, Northern Parts of Ceylon (Hooker, 1874; Duthie, 1903). Duthie (1903) has also reported that the plants are plentiful within the area especially in sandy and rocky places. Kirtikar and Basu (1935) have described its distribution upto 3,385 metres (11,000') in Kashmir. Following species have been reported in India.

1. Tribulus terrestris Linn.,
2. Tribulus longipetalis Viv.,
3. Tribulus lanuginosus, Linn.,
4. Tribulus subramanyamii Singh,P.,
5. Tribulus rajasthanensis Bhandari,
6. Tribulus cistoides, Linn.,
7. Tribulus alatus Del.Fl.,

It is relevant to give brief description of these species.

A) Tribulus terristris :

a) Distribution :

Out of these 7 species Tribulus terrestris is a cosmopolitan species and is distributed throughout India ascending to 3,385 metres (11,000') in Western Tibet, northern part of Ceylon (Hooker, 1874; Duthie, 1903).

According to Rendle (1952) the plant is a native of dry tropical South Africa. Cooke (1958) has reported it from

dry and hot parts of Bombay presidency as a common weed and has also described it as member of desert flora. Gamble (1958) has recorded the plant chiefly from dry hot localities of Deccan. Blatter and Halberg (1918-21) have reported it from different region of Jodhpur and Jaiselmer in Western Rajasthan. Puri (1952) has reported this plant as one of the typical gravel plants from Loharki and Bap to Jaiselmer while Sarup (1952) has recorded it from Jodhpur and North Western Rajasthan. Biwas and Rao (1953) have mentioned this is a desert plant. The other workers who have reported the plant are Joshi (1956) and Sarup (1958). Vyas (1958) reported from mixed sand and rocky areas, sandy plains and sand dunes, and loamy plains and loamy dried up soils of Jodhpur Tehsil.

In Eastern Rajasthan Ratham (1951) from Lohargal Ratham and Joshi (1952) and Bakshi (1954) from Pilani Nair (1956) from stabilized soil of Chirwa, Joshi (1967) from plain areas of Shekhawati, Joshi (1958) from stabilized dune areas of Pilani, Nair and Nathwat (1957) from Sandy plains of Harshnath. Arvalli hills, Nair and Kanodia (1959) from the plains of Ajit Sagar Bundh. Nair et al. (1961) from Khetri town and Nair and Malhotra (1961) from Lohargal are the authors to record and report the plant.

b) General morphology of Tribulus terrestris L. :

Tribulus terrestris is a procumbent branched herb forming large circular patches on the ground by the

characteristic development of the lateral branches (Plate 2).

The plants usually grow in moist open places stabilized areas and dunes and is very commonly found on either side of the road and near the fencing walls as a rainy season weed.

The plant possess normal tap root system which varies in length number of laterals and other ramifications from habitat to habitat. There is quite dense net work of secondary and tertiary branches which ramify in all directions of various level. The deeply seated extensive roots often have equally stout laterals which ramify further. A cylindrical solid with distinct swollen nodes and pilose internodes (1.04.5 cm in length). Internodes thin and elongated in dwarf plants. Stem is covered with white hairs, branches vary in length in different localities and may even reach a length of more than a meter in certain cases.

Stipulate (lanceolate) pinnately compound generally paripinnate but imparipinnate leaves have also been observed. Leaves are alternate in the lower region while opposite in the upper. Each leaf has 3-7 pairs of leaflets which are sub-equal mucronate, oblong base round and oblique entire margin obtuse apex. Venation reticulate unicostate and are covered with silky hairs on both sides. Longer on the lower side thus rendering it more shining leaflets of the dwarf plants are smaller than those of healthy plants.

Ebracteate, pedicellate (pedicels 1.2-2.0 cm long slender and hairy) complete bisexual actinomorphic usually pentamerous (but hexamerous flower also found) hypogynous, yellow in colour and 1.2-2.0 cm in size (Plate3).

Sepals 5 measuring 0.5-0.6 cm in length and 0.3-0.4 cm breadth polysepalous yellowish green in colour, lanceolate hairy on the outer surface deciduous thin margin quincu n cial aestivation.

Corolla petal 5 (6 in hexamerous) 0.5 cm long polypetalous spreading and fugacious. Each petal is obovate or oblong with a distinct but short claw. Twisted aestivation. Androecium : stamens 10 (12 in hexamerous) arranged in two whorles a condition recalling obdiplostameny. These are alternately longer and shorter. The shorter ones have small glands outside and are all inserted on the base of the ten (02-12 lobed in hexamerous) angular disc. filaments yellow and filiform slightly flattened at the base. Anther dithecous dorsifixed and yellow. Pollen grains are round with reticulate exine (Agabqyan,V. et al., 1966).

Gynoecium pentacarpellary syncarpous superior hirsute ovary style is stumpy and short stigmatic lobes 5 ovules many axile placentation.

Fruits globose, woody and spiny capsule. Indehiscent with usual number of five cocci (average length breadth and weight 0.70, 0.40 cm and 0.0306 gm respectively (Plate 4).

Each Coccus has two pairs of hard and sharp spines one pair is longer than the other. The divergent spines are the direct outgrowths of the pericarp which help in the dispersal of seed. The number of seeds per coccus is as many as 4 with transverse portion between them but cocci with minimum 3 and maximum 7 seed are also found.

Seed colour is white to yellowish but some times these are greenish white also. This possesses a round margin with slightly elogated pointed end. The average length breadth and weight of seed is 0.376 cm, 0.150 cm and 0.00115 gm respectively. (Plate 5).

c) Phenology : The plants makes its appearance in the month of July after receiving the first few showers of rain. It takes hardly 2 to 3 weeks to flower from the time of its sprouting. By the end of July and in August most of the spots in the area are seen covered with Tribulus terrestris giving a yellow look to the ground due to its yellow flowers. The flowering season lasts up to November, although the fruiting starts from the middle of September. It is found that the plant reaches its Zenith in the following two months. Many plants survive throughout the winter and can be found even in the month of February at certain favourable spots. Indehiscent cocci get separated from one another and fall on the ground after the capsules are completely dried on the plant in month of October to November. Some of the plants are seen

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perennating by their root stocks and produce aerial parts again after receiving few or occasional winter rainfall. These plants last even upto February end.

d) Uses : Plant and dried spiny fruits are used in decoction or infusion in case of spermatorrhoea phosphaturia, diseases of genito-urinary system such as dysuria, gonorrhoea, gleet, chronic cystitis, calculous affections, urinary disorders, Kumarz,G. et al. (1967), incontinence of urine, gout and impotence; also in uterine disorders after parturition and to ensure fecundity and used in Northern India in cough, diseases of heart and suppression of urine. Water rendered mucilagenous by the plant is drunk as a remedy for impotence and an infusion of the stem is administrated in gonorrhoea. It is generally given with hyoscyamus and opium in inflammatory conditions of the urinary passages. Chakradatta recommends a decoction of the fruits with the addition of impure carbonate of potash to be given in painful micturition. The fruits form an ingredient in medicines for urinary disorders and impotence and is one of the ten ingredients of the 'Dasmula-Kvatha' a compound decoction often mentioned in Ayurveda. A compound powder called 'Gokshuradi Churnam' is popular in all urinary diseases which is made up of Tribulus terrestris. Nine tolas cubebs, Mesua ferrea, Rhel radix and potassium nitrate each 3 tolas powder and mix. Dose is 10 to 20 grains. This drug was given a good trial in cases of Bright's diseases

with dropsy. All the patients derived much benefit by its use. It was also combined with bdellium in a patient suffering from gonorrhoeal rheumatism with cystitis. The patient recovered without interruption (Ind. Drugs Report, Madras). A decoction of the entire plant is given with silajatu and honey in the same affection. Equal parts of Gokhru and sesamum seed taken with goats milk and honey cures impotence arising from vicious practices. Bhavaprakasha give the composition of an eletuary known as Gokshuradyava leha recommend in painful micturition, suppression of urine, bloody urine, calculous affections etc.

Sushruta prescribes the root in combination with other drug in the treatment of scropion sting but it is not a antidote to scorpion venom (Caius and Mahaskar). In China the fruit is used as a reputed tonic and astrigent. It is used for coughs, spermatorrhoea scabies, anaemia, ophthalmia it is powerful hemostatic, much used in postpartum haemorrhage and in dysenteries. In South of France and in the Southern countries and Europe the roots and the leaves are considered tonic and aperient.

B) Tribulus alatus : Hindi - Gokhurikalar, Urdu - Bakhta.

a) General morphology :

Annual branches prccumbent or ascending densely silky-villous. Leaves as in the former species, stipules broadly ovate acute hairy flowers 8-12 mm diam. sepal 4-5 mm long

ovate acuminate, villous cutside. Petal very thin scarcely longer than the sepal cuneta oblong, the apex sometimes slightly denate stamens 5. Ovary bristly with long white hairs style short stout fruits of 5, 2-seeded, cocci, the cocci very hirsute the spine confluent in to toothed wings.

b) Uses : The fruit is a good appetiser good emmenagogue, cures inflammation useful in uterine complaints (Ayurveda). In Baluchistan the fruit is a domestic remedy for uterine disorders after parturition.

C) Tribulus rajasthanensis :

a) General morphology :

Perenial or rarely annual, diffusely prostrate or somewhat ascending herb, stems many from some what woody root-stock upto 3 dm. long densely pubescent and sparingly hirsute glabrescent striate leaves 2-5 cm long opposite. Sepal 6-7 linear lanceolate acute margins, scarious villous outside. Petal 6-8 broadly obovate cuneate stamen 8-10 filament 3-4 mm long anther 1.5-1.7 mm long ovate oblong subequal ovary with bulbous based bristly hairs directed up words style upto 3 mm long.

3. Zygophyllum : In this genera only two spp. are medicinally important (Kirtikar and Basu, 1935).

1. Zygophyllum simplex Linn.,
2. Zygophyllum coccineum Linn.

A) Zygophyllum simplex Linn.Mant. :

a) General Morphology :

A succulent watery much branched herb branches slender, reddish striate, glabrous, Leaves small subsessile, cylindric, oblong or obovate, obtuse. Fleshy stipules lanceolate acute flower small sepals obovate cucullate of the apex, petal yellow spathalate, spreading margins. Flat staminal scale bipartile hyaline the segments obovate. Ovary turbinate glabrous ribbed style tapering, capsule deflexed, rugulose, separating into 5 compressed 3-5 seeded cocci, seed minute, oblong attenuated at both ends smooth.

b) Uses : The Arabs beat up the leaves or the seed in water and apply the infusion to the eyes in ophthalmia and leucoma. They also consider the seed anthelmintic.

B) Zygophyllum coccineum Linn. :

a) General Morphology :

Suffruticose, branches woody striate and papillose houry at first with a white powdery tomentum at length glabrous. Leaves 2 foliate powdery, petiole stout fleshy, grooved, longer than the leaflets, stipules triangular, scarious, leaflets oblong. Cylindric or semicylindric, obtuse minutely powdery. Flowers solitary, white or tinged with yellow. Sepal fleshy cucullate the margins membranous, petals spathulate spreading, longer than the sepals undulate on the margin. Staminal scale

ovate lanceolate, entire or lacerate at the apex ovary glabrous or velvety capsule 1 cm. long oblong or abovoid, cocci 3.5-seeded, seed small ovia acute tubercled, compressed.

b) Use : The seeds are reputed anthelmintic among the Arabs.

4. Fagonia : In this genera only one spp. is important.

A) Fagonia cretica :

a) General Morphology :

A small spiny undershrub with stiff branches often more or less prostrate. Twings slender, terete, striate glabrous gladular leaves opposite 1.3 foliate, about 12 by 2.5 mm entire linear or elliptic mucronate petiole. Very variable 0.3 cm long sometimes leaf like, stipules transformed in to sharp slender. Spines upto 1.2 cm long persistent and continuing growth long after the fall up the leaves. Flowers solitary rose coloured on penduncle 5-12 mm long arising from between the stipules. Sepal 5 deciduous imbricate half as long as the petals. Petal 6 mm long spathulate with a marked claw disk short inconspicuous. Stamens 10, inserted on the disk, filament filiform naked anthers oblong ovary hairy sessile 5-angled, 5-celled, tapering into 5-angled style stigma simple. Fruits 5 mm long of 5 seeded cocci glandular pubescent deeply 5-partite almost to the axis cocci dehiscing along the ventral seuture and separating from a horney endocarp.

b) Uses : The plant is acrid and bitter, colling removes 'vata' asthma fever, thirst vomiting; cures, fevers, dysentry urinary discharges erysipelas, typhoid, alexipharmac reduce tumours, purifies the blood. Sushruta presdribes the leaves and twings in combination with other drugs for the treatment of snake bite.

The plant is useful as an application to tumours also in chronic fever, dropsy and delirium and in any disorder which arises from poisoning. It is largely used by the native practitioners as a bitter and astrigent tonic.

5. Cytology : The perusal of the literature revealed that the family zygophyllaceae has been fairly well studied as they provide ideal material for cytological investigation. The knowledge of chromosome shape and size in related taxa can be of fundamental importance to the evolutionists and taxonomists.

Genotype and phenotype analysis are two ways of studying the phylogeny of plants and animals. The genotype analysis deals with genetical and cytological analysis method and their carrier, linkage groups and genome. Whereas the phenotype analysis concerns with menifestation of the characters. It is a method of approach of systematists and morphologists. The inseparable relation between genes and chromosomes has been known in plants and animals and particularly since the discovery of salivary glad chromosomes and function of drosophila

(Heitz, 1925). Accordingly the chromosome morphology has been believed to be one of the important characteristics to discuss the phylogeny. This new method is a karyotype analysis. In the karyotype analysis chromosome number (especially 'n' number) morphology (including length and breadth of chromosome) of chromosome, position of primary and secondary constriction must be carefully examined and determined. The morphology of chromosome set consisting of a basic chromosome number (n) namely basikaryotypes (Sato, 1939; Sinoto and Sato, 1940) is the most important characteristic of the karyotype. By means of karyotype analysis we detect certain combinations of the basikaryotypes in the material examined and we can also see resemblances or alteration of the basikaryotypes. The resemblance of karyotype is presumed to indicate the phylogenetic resemblance between them.

In the case of meiosis the pachytene stage of meiosis in many respect is ideal for the study of chromosome morphology. Pachytene analysis has been made by several investigators in variety of species. Such studies were carried out in zygophyllaceae (Bhansali and Bhandari, 1974),

Pachytene analysis is particularly rewarding in species with short chromosome. The relative length of the pachytene stage of meiosis gurantees a high number of cells for analysis.

The cytological study of the family zygophyllaceae has been carried out by the number of workers (Darlington, 1939; Negodi, 1947; Bhansali & Bhandari, 1974). The sub-chromosome number has been reported to be X = 12 with 2n = 24 chromosome while Schnack (1947) reported 2n = 48. Bhansali & Bhandari (1974) reported chromosome numbers of some Indian desert plants. In this work they also reported the chromosome number of some zygophyllaceae member Tribulus alatus n =12, Tribulus cretica n = 10.

In 1979 Gerald reported chromosome no. of some flowering plant. In this case a zygophyllaceae member included is Tribulus cistoides L. n = 18. Zygophyllum is another genus of the family and chromosome number reported is in Zygophyllum fabago x = 11 (Warburg, 1938). Fagonia is one of the genera of zygophyllaceae which has been worked out : Fagonia cretica has x = 9 (Negodi, 1939). Bhansali and Bhandari (1974) in their report listed chromosome number of Fagonia criteca as n = 10.

Kumar (1978) studied Intraspecific hybrid and polyploidy in Tribulus terrestris occurring naturally with chromosome number of n = 18 and 24.

6. Mineral nutrition :

The comples structure of secondary products made difficult to study the biogenesis of these compounds and the enzymes

involved in the system. Nevertheless the study of biogenesis of cyclic amino acids and such other complexes primary compounds have been known and the important minerals involved as a cofactor are also known. Gasic et al. (1978) extensively serveyed the colchicine content from Colchicum autumnale in relation to soil mineral composition. They established the positive correlation between the trace element Cu, Zn and Mn and the total alkaloid content. It is well established in the literature that aromatic amino acids such as phenylalanin, tyrosine and tryptophan play a pivotal role in metabolism of large number of complex compounds including alkaloids (Fowden, 1965). Zinc is one of the important trace element which is known to have a role as cofactor in tyrosine synthesis and Mn has the same role to play in tryptophan synthesis.

Nicholas (1961) Bennett and Heftmann (1969) and Capstack et al., (1965) have shown that in plants Mavolonic acid is converted to squalene and later to sterols Capstack et al. (1965) demonstrated that the squalene formed in plants had same labelling pattern as that of animal. Mavalonic Kinase and Phosphomavalonic kinase have been shown to the first two enzymes involved in the conversion of mavolonic acid to the mavolonic 5-pyrophosphate through mavalonate 5-phosphate. Both of the reactions are known to require ATPase phosphate donor and Mg^{++} or Mn^{++} promote greater enzyme activity (Techen, 1958). In the activation of the enzymes low concentration

Mn^{++} are more effective than Mg^{++} (Techen, 1958; Williamson and Kekwick, 1965). This foregoing brief scanty information provides a clue that some of the trace elements control the metabolism of secondary compounds.

7. Flavonoid & Glycosides :

Genus Tribulus is one of the well-known genera for medicinal value. Several species of Zygophyllaceae have been investigated for flavonoid glycosides (Chirikdjian, 1973). Bhutani et al. (1969) were the first to work out the presences of flavonoid and phenolic constituents in Tribulus terrestris. This work was mainly carried out by them not to work out on identifying these compounds for their medicinal value but because of photosensitivity. According to them number of workers have recorded the presences of saponon, Kinitia et al. (1972), Tomowa et al. (1978); diosogenin, ruscogenin, gitogenin etc. and attributed the photosensitivity to them (Tomowa et al., 1977). However, the main objective of Bhutani et al. (1969) in investigating the flavonoids is to study phenolic constituents to which the flavonoid belongs. They develop technique separating the main generic compounds of flavonoids in crystalized form. They found that ether and ethyl acetate fractions are identical and give positive colour reaction for flavonoids. This technique of chemical fraction was eventually combined with chromatographic technique on silica gel and they were

successful to identifying on the TLC Kaempferol (K)-3-glucoside, Kaempferol-3-rutinoside and tribuloside. The fourth compound Tribuloside which has been found in large quantity in Tribulus terrestris has also been shown a derivative of (K) Kaempferol. These compound have been extensively studied for their physical and chemical properties by application of the technique of NMR spectroscopy. Even structure of Tribuline has been determined.

After Seshadri school the broad out line of flavonoids of Tribulus terrestris L. in 1969 till 1982 no work on these aspect has come out. In 1982 N.A.M.Saleh et al., (1982) worked out at depth of the flavonoid glycosides of two species of Tribulus viz. T.pentandrus and T.terrestris. By using various techniques such as chromatography, UV spectrometry, enzymatic hydrolysis, chemical fractions of 25 different flavonoid glycosides in leaves of Tribulus terrestris and T.pentandrus were identified beside (K)-Kaempferol some of flavonoid hitherto not describe are (Q) Quercetin, (I) Isorhamentin, (T) Tricin this gives spectrum of varieties of flavonoid glycosides found in the leaves. However, they did not identify the flavonoid glycosides of seed.

8. Biogenesis of diterpene :

Diterpenes are widely distributed through out the plant and animal Kingdom in exceptionally diversified form. Though for the years the word 'terpene' has become associated with

fragrant, steam volatile substances from higher plants with the advent of modern biochemistry and recognition of the significance of isoprenoid structure to animal physiology this association has diminished (Nicholas, 1967).

Studies on terpene biogenesis in plants has been accelerated by the use of the ^{14}C isotope technique and the discovery of mevalonic acid (Trease and Evans, 1972). The pathway for the formation of Alkaloids have been proposed for many years. Although many hundreds of individual alkaloids have now been characterized the investigations to date indicate that majority of nitrogen units of the alkaloids are derived from a relatively small group of amino acids with acetate - mevolanate and methyl donors. These small carbon skeleton have considerable role to play. Many pharmacologically active alkaloids arise from various amino acids (Trease and Evans, 1972).

i) Orinthine derived alkaloids,

ii) Lysine derived alkaloids,

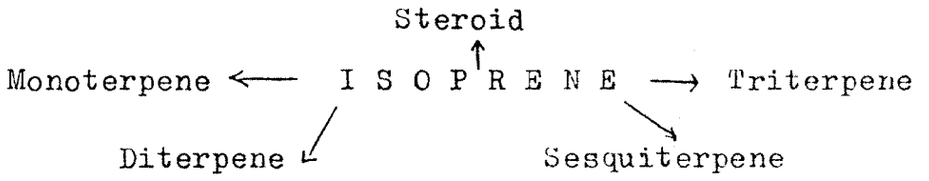
iii) Phenylalanine, tyrosine and Dihydroxy - phenylalanine derived alkaloids,

iv) Tryptophan derived alkaloid.

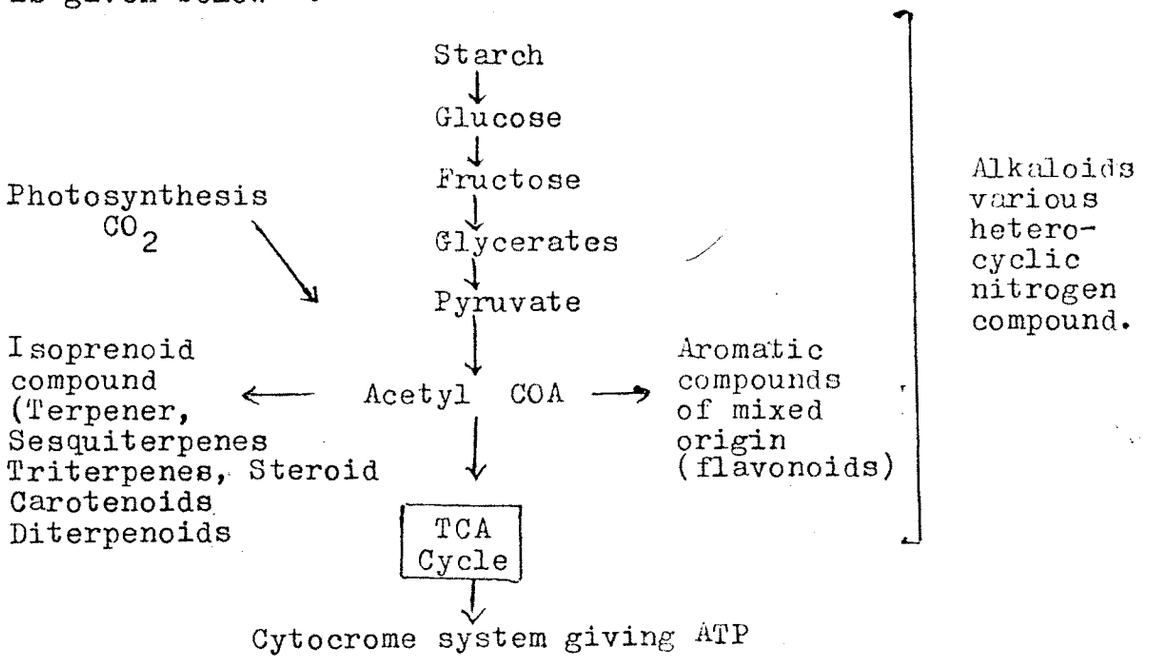
9. Isoprenoid Compounds :

As result of the extensive pioneering investigations in to plant terpene structure Ruzica (1953) published his

biogenetic Isoperene Rule. This indicated that application of isoprenoid units could be used to explain the formation of monoterpenes, diterpenes, sterols and triterpenes with complex constitutions.



Some basic metabolic pathways appear to be similar in both plants and animal (Trease and Evans, 1972) whereas other are more restricted in their occurrence. It is to the secondary plant products the majority of vegetable drugs owe their therapeutic activity. The production of these secondary metabolites is dependent on the fundamental metabolic cycles of the living tissue including photosynthesis from which carbon skeleton is derived. The broad out line of the metabolic links is given below :



Adapted from Trease and Evans, 1972.

The discovery of acetyl CoA in 1950 the so called active acetate gave support to acetate in biosynthetic processes. The next major advance in this field was the discovery in 1956 of mevalonic acid (3,5 dihydroxy 3-methyl valeric acid) and the demonstration of its incorporation. Mevalonic acid is the 6 carbon acid which forms the basic building block of the isoprenoid compounds. During next four years research involving the use tracer technique, inhibitor studies, cell free extracts, partition chromatography ionophoresis and synthetic organic chemistry led to establish that the 5-carbon compound for which biochemists had been seeking so long was isopentenyl pyrophosphate. It is derived from mevolonic acid pyrophosphate by dehydration an carboxylation. Isopernoid synthesis then proceeds pyrophosphate to yield geranylpyrophosphate. These priliminary stages in the biosynthesis of isoprenoid ccmpounds an envisaged by Lynen and cornforth are shown as below :

Acetyl - CoA

↓

Acetoacetyl CoA ⟶ Hydroxymethyl glutarate

↓ 2 TPNH

Mevalonate

↓ ATP

5-Phosphomevolonate

↓ ATP

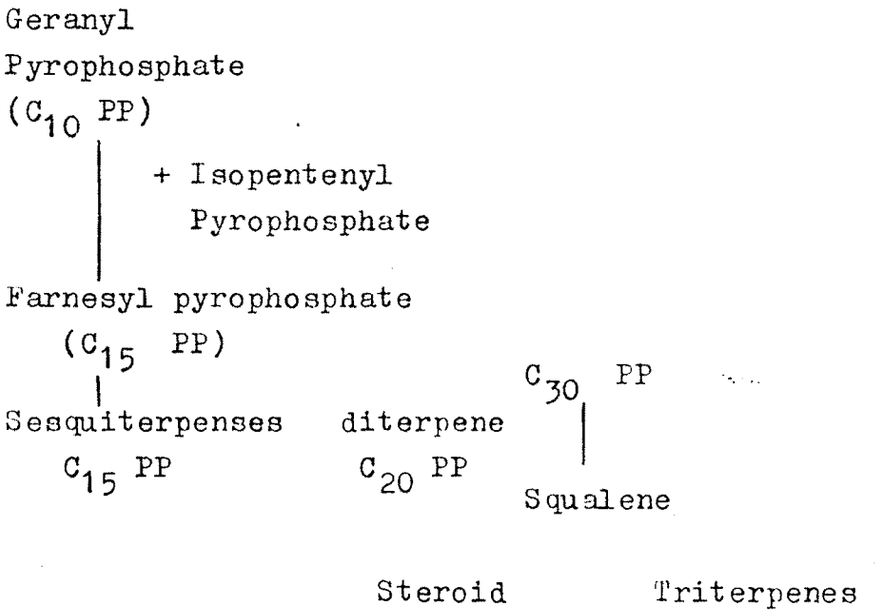
5-pyrophosphomevolonate

↓ ATP - CO_2

Isopentyl Pyrophosphate

↓

Dimethylallyl pyrophosphate



(Adapted from Trease and Evans, 1972).

10. Cultivation of medicinal plants :

Use of plants and plant parts for the purpose of medicine has been in practice in India from the Vedic time. The very Ayurvedic principle is based on this even in the middle East and Europe. From the Egyptian time the plants were variously used for medicinal purpose. Similar to that of Ayurveda, Unani literature also deals with the same.

However, the medicinal plants in those days were being tapped from natural resources. The systematic survey of natural products of India mainly the medicinal plants were carried out during the British time by George Watt. The cultivation of

medicinal plants aromatics and etheral oil yielding plants, were started in mediterranean countries for the purpose of systematic study and development of industry. In India it has never been practiced either by government organisation or by any private organisation. The multinational pharmaceutical companies have only exploited the natural resource rather than undertaking cultivation of them. A few companies such as Roussel Pharmaceuticals have undertaken wide range cultivation of some medicinally important plant such as Gloriosa. However, many of the other multinational pharmaceutical companies have not take up possibly, due to the lack of necessary systematic study. In Europe and in Russia and Hungery the line of thinking have changed and advanced. They are thinking on the line of collecting culturing, stocking and distribution of plants (Tetenyi, 1988).

Certain drugs are now obtained almost exclusively from cultivated plants. These includes cardamoms, Indian hemp, Ginger cinchona and opium. Flax, poppy and cocoa have been cultivated from time immemcrable. To meet the demand wild plants are cultivated. Climatic factor such as temperature, rainfall, day length, altitude and soil condition affects the plant growth.

Experiment carried out in the phytotron at Fif.Sur. Yvette indicated that with Datura tatula long exposure to intense light brought about a sharp increase in hyoscine content

at the time of flowering. In long dry conditions papermint leaves contain menthone, menthol and traces of menthofuran. Plant grown under short day conditions contain menthofuran as a major component of volatile oil. In the case of Cinchona succirubra the plants grow well at low altitude level but produce practically no alkaloid. Rainfall also have a great influence on vegetation.

If the soil differed from each other it induces difference both in physical and chemical properties. Fine soil rich in humus and having a permeable substratum is generally favourable for plants.

11. Preparation of Soil :

Land is cleared and tilled. Moisture content in the soil is regulated by adding humus "Farm Yard Manure"Irrigation, tillage operation is done to assist drainage and to loose soil, suitable for germination of seeds and free growth of root. The fertility of soil is restored by adding suitable doses of fertilizers and manures containing nitrogen, potassium and phosphate or lime. The farmyard manure is a general manure in that it contains all the elements required by the plants. If the soil is deficient in micronutrients the analysis of soil is done and deficient micronutrients are added. Some time impoverished soil is kept fallow to resort its fertility. Some times the intercroping is done with leguminous crops for increasing nitrogen content of the soil.

12. Propogation : Plants are reproduced either from seed or by vegetative means, using cuttings, tubers, rhizomes or any other underground or areal parts.

a) From seeds : To ensure success in germination seed must be perfectly ripe air dried and fresh. Before, sowing germination percentage is tested. If seeds have hard coat they are scarified by treating with hot water or sulphuric acid. The small seeds are mixed in the soil at the time of sowing. Generally spreaking seeds may be burried to the depth of their smallest diameter, but as a protection against birds etc. It is some time advisable to sow them deeper.

b) Vegetative method of propagation : Vegetative propogation is the rule in some plants. The plants with higher genetic variability and heterozygosity can not produce viable seeds. Therefore, they usually reproduce by means of vegetative propogation. For vegetative propogation various types of plant parts are used such as bulb, corm, tuber, rhizome, root stocks, stem cuttings, runners, suckers etc. For propogation plant part is used carefully. It is always better to select healthy disease free, soft and fresh material. For easy rooting in case of cuttings plantation is done in equal part of sand and peat and rooting medium is added sometimes. Sometimes vegetative propogation is done in glass house for maintaining temperature and humidity. Afterwards they are transplanted in the field, grafting, layering and budding method is also applied in most of the times.

In case of microcrganisms such as bacteria and moulds for manufacture of antibiotics fermentation method is used. Also for valuable organisms agar nutrient is used for multiplication.

To facilitate the proper growth, the growth regulators such as Auxins, GA, Cytokinin, Abscisic acid, Ethylene is used in proper concentrations.

Weed killers or herbicides (selective or non-selective) such as mercuric chloride, sodium chloride, Auxins and 2:4 dichlorophenoxyacetic acid are applied for good healthy growth.

In India, Central Drug Research Institute, Lucknow, National Botanical Research Institute, Lucknow, have been working on different mediciral plants and their active principles as well as cultivation. But maintenance of medicinal plants on large scale is scanty. Hoechst Pharmaceutical Company which has for the first time detected the hypersensative principle in Coleus is also not maintaining the large scale cultivation of Coleus forskohlii.

13. The collection drying and storage of drugs :

Pharmacognosy is concerned with both the living plants and the dried drug prepared. Many plant constituents are relatively stable and therefore, occur both in fresh plant and in the dried drug. Others may undergo changes brought about by enzyme heat and moisture during drying and extraction

processes. Sometimes, these changes are very complex and condition must be strictly controlled to give the product desired. Familiar examples of this are the preparation of tea, cocoa and tobacco.

In plant breeding and the scientific control of the cultivation collection drying and storage will do much to improve the quality of the medicaments derived from it. The season at which each drug is collected is usually a matter of considerable importance. Sometimes the nature and amount of the active constituents is not constant throughout the year. The age of plant also affect the quality and preparation of the drug in active mixture. There is increasing evidence that the composition of number of secondary plant metabolites varies appreciably throughout 24 hour and is studied.

a) Collection : In plant breeding and the scientific control of the cultivation, collection, drying and storage of drugs will do much to improve the quality of the medicaments derived from the studies on the collection of Podophyllum, Ephedra, wild cherry and aconite have shown that the season at which drug is collected is an important factor. The plant rhubard contains anthranols in winter and are converted by oxidation in to anthraquinones. Betts and Fairbairn (1964) have shown that the contents of C-glycosides, O-glycosides and free anthraguinone in the developing shoots

and leaves of Rhamnus purshiana, fluctuates markedly throughout the year. The age of plants also affect quality and proportion of the active constituents in the active mixture. From the studies in Digitalis, Pinus, Poppy and Salvia, it has been reported that there is interconversion of the various alkaloids of glycosides present in it.

b) Drying : Before drying plant part should be free from disease. Drying should be done slowly at a moderate temperature since drugs containing volatile oil are liable to lose their aroma. Fresh drug contains 60 to 90% of water. In drug such as Clove, Cardamon and Cinnamon open air drying is used. Drying by artificial heat is more rapid and suitable heat may be applied by open fire or hot water pipes. For rapid drying at low temperature a vacuum dryer may be used. Fairly rapid drying helps flowers and leaves to retain their colour and aromatic drugs, their aroma. The temperature used in each case must be governed by the constituents and the physical nature of the drug. As a general rule leaves, herbs and flowers may be dried between 20° to 40°C and barks and roots, between 30° to 65°C.

Exactly how far drying is to be carried out is a matter of practical experience. For drying purpose generally trays of variable size are used also suitable permanent construction is also done.

c) Storage : Drugs are frequently examined during storage. Drugs are stored in the usual containers such as sacks, bales, wooden cases, cardboard boxes and paper bags which can reabsorb 10-12% or more moisture. Drugs such as digitalis and Indian hemp are never allowed to air dry so that they may be kept in a sealed containers with dehydrating agents. For large quantities the bottom of case may be filled with quick-lime and separated from the drug by perforated grid or sacking. If the time becomes moist it should be renewed. Volatile oils should be stored in sealed, well filled containers in a cool place, sometimes air in the container is replaced by an inert gas.