Chapter 1
INTRODUCTION

1.1 Occurrence and distribution of polyphenols in nature.

Polyphenolic compounds include groups of complex natural phenols which are mostly polyhydric and are very often oxygen heterocycles. They are frequently coloured substances and constitute much of the pigmentation in nature and therefore widely distributed in plants. They are classified as the secondary metabolites produced in plants (Ilhaslam, 1989). In plants they are variedly distributed and the polyphenols vary from plants to plants. Many polyphenolics occur in living cells in combined forms as glucosides and esters. In addition to natural polyphenols such as simple monocyclic phenols, chromones, depsides, quinones, coumerins, lignans, benzophenones and xanthones and ilvanoids, lignins and tannins are important groups of polymeric polyphenolic materials of the class of phenolics that occur in combination with living cells. The term tannin dates from 1796 when it was first used (Seguin, 1796) to denote substances present in plant extract which possessed the property of converting animal skin to leather.

The results of several of researches quoted earlier have indicated the probable region of maximum accumulation or deposition of polyphenols in certain species. The work on the tannins of 'Quercus' species involving ringing experiments led Hathway (1959) to the conclusion that the pyrogallols formed in the leaves are translocated downwards by the sieve tube system to the cambium where they undergo oxidation through quinone, by the cambium polyphenol oxidase in the outer bark. However an explanation in better accord with the the recently obtained data is
that sucrose and related Oligosacharides are translocated and are converted insitu (Hillis, and Carle, 1960) together with the stored carbohydrate into the relevant polyphenols by the local enzyme system.

There are many factors that affect the rate and amount of accumulation of tannins and other polyphenols in the barks and wood of forest trees. Investigations show that the polyphenol content varies with the nutrition of the soil, wetness and dryness of soil, mild climate etc (Vogel, 1931). The bark of trees of *Acacia* species growing in high rainfall areas has been found to contain less tannin than that from trees grown in a lower rainfall area (Sherry, 1952). The height factor has been quoted for black wattle bark, that near the top of the tree having appreciably less tannin than the bark (Williams, 1930). Sunlight plays an important role as a stimulant in the formation of the polyphenols in the plants (Hillis and Swain, 1959). A higher potassium content also helps in the production of polyphenols in plants. The *Sirex* unattacked sapwood of *Pines radiata* tree contains very small amounts of polyphenols, whereas the one which is attacked by *Sirex* is notable in the content of polyphenols (Hillis and Inoue, 1968) in the sapwood cells of the plant.

### 1.2 Bio-synthesis of polyphenols in plants

Polyphenolic compounds arise essentially by one of the three pathways: (a) the acetate-malonate pathway (b) the Shikimic acid pathways (c) combination of the both (the mixed pathway). Feeding experiments with labelled precursors indicate which of these pathways is operative in a given case. Recently the individual steps of the biosynthesis have been elaborated in a number of polyphenols by the realization of respective transformations by the isolated enzyme. It should be noted that
the various pathways could operate for the production of a class of polyphenols or even a single compound up to the organism concerned. From comparative view point, the acetate-malonate pathway is more common on bio-synthesis in microorganisms and the shikimate pathway is more popular for higher plants.

(a) Acetate malonate pathway (Turner, 1971)

The primary building block in this pathway is the linear poly-β-keto compound produced from an acetate and several malonates by a process analogous to fatty acid bio-synthesis. The presence of poly-β-keto compounds as precursors in polyphenolic compound biosynthesis is greatly strengthened by the isolation of acyclic polyketides, triacetic and tetracetic lactones from a strain of *Penicillium stipitatum* (Acker et al., 1966).

(b) Shikimic acid pathway (Haslam, 1974)

A wide range of polyphenolic compounds originate from the essential amino acids, phenylalanine, tyrosine and tryptophane or the intermediates involved in their bio-synthesis from glucose, in which shikimic acid plays a key role.

1. $\text{C}_6\text{-C}_1$, $\text{C}_6\text{-C}_2$, and $\text{C}_6\text{-C}_3$, compounds (Pridham, 1968)

$\text{C}_6\text{-C}_3$, and $\text{C}_6\text{-C}_1$, compounds oxygenated at 3,4,5 portions (eg. $p$-hydroxycinnamic acid, syringic acid, procatechuic acid, sinapic acid) are very common constituents especially in higher plants. $\text{C}_6\text{-C}_2$ (acetophenones and phenyl acetic acids are less common. Most of these compounds derived from phenylalanine and tyrosine through deamination followed by hydroxylation on the ring or degradation of the side chain or other modifications. These processes have been studied closely from the ground of enzymology (Hanson and Havir, 1972).
In several cases the direct production of some of these compounds from shikimic acid has been demonstrated (Dewick and Haslam, 1968). The formation of procatechuic acid and gallic acid is closely related to the biogenesis of some tannins (Swain, 1965).

2. Coumarins (Brown, 1966 and Neish, 1965)

They are synthesised from trans-cinnamic acid via sequence of the reactions: ortho-hydroxylation, glycosylation, stereomutation and cyclization.

3. Lignins (Nord and Schubert, 1967) and Lignans (Pridham, 1965)

The biosynthesis in lignins in general is by the radical polymerisation of cinnamic alcohols like trans-para-hydroxy cinnamic alcohol, trans-coniferyl alcohol and trans-sinapyl alcohol which are derived from the corresponding cinnamic acids. Lignin biosynthesis is supported by the polymerisation of phenyl propane monomers or peroxidases.

Lignans comprise the dimers of all types formed by the oxidative coupling of p-hydroxyphenyl propane units (McCredie, et al, 1969).

(c) Mixed Pathway

1. Xanthones: The result of labelling experiments (Gupta and Lewis, 1971) seem to favour the proposal that Xanthones in higher plants are biosynthesised by the direct oxidative coupling of the hydroxybenzophenones derived from acetate and shikimate (Farkas and Pallos, 1967).
2. Flavanoids (Grisebach, 1965): The key compounds in the biosynthesis of flavanoids are chalcones. The interconversion between chalcones and flavanones occurs easily and these are converted to various flavanoid compounds by oxidation, reduction or numerous other secondary transformations. Polymerisation of catechins and flavan-3,4-diols lead to the formation of some sort of tannins (Weinges et. al, 1964).

3. Isoflavonoids (Grisebach, 1975): It is established that the isoflavones are formed by the 1,2-aryl migration of the corresponding flavones.

4. Neoflavonoids (Seshadri, 1972): A novel group of interesting compounds which have the carbon skeleton of 4-phenylcoumarin have been demonstrated to arise by nucleophilic attack of a phloroglucinol nucleus on a cinnamyl residue at the α-carbon of the side chain with the elimination of a suitable leaving group.

5. Stilbenes (Billek and Shimpl, 1966): The β-polyketo intermediates responsible for chalcone formation when subjected to another cyclisation (aldol type) with subsequent decarboxylation give stilbenes.

1.3 Functions, Physical, Biological and Chemical properties of polyphenols in nature.

The polyphenols in nature mainly accounting for the ones found in plants due to its wide distribution are found in the bark, leaf, flower and in fruits of plants. While some polyphenols render pigmentation, some others protect the plant from the invasion of insects and microorganisms that destroy them.
The production of polyphenols after the attack of wood-wasp *Sirex noctilio* on *Pinus radiata* is responsible for the recovery of the tree from subsequent drying up (Hillis & Inoue, 1968). Thus the tree is protected from the attack of the wasp by the production of polyphenols.

Certain tannins as reported by Haslam (1996), serve as drugs and if not properly maintained in the dietary intake can cause several diseases. It is also pointed out that there is a reduced risk of degenerative diseases by the consumption of beverages containing polyphenols in particular green tea and red wines both rich sources of polyphenols (Waterhouse, 1995).

There are many medicinal plants which have polyphenolic metabolites as given by Haslam (1996) and they are:

1. **Tree peony** (*Paeonia lactiflora*) - Outer skin of the root; to cure disorders of blood stream. Principal polyphenolic metabolite - gallotannins.

2. **Bear Berry** (*Arctostaphylos uva-ursi*) - dried leaves; to cure ailments of bladder and urinary tract. Principal polyphenolic metabolite - gallotannins.

3. **Rasp Berry** (*Rubus idaeus*) - leaves and fruits; to cure digestive disorders. Principal polyphenolic metabolite - ellagitannins.

4. **Hawthorn** (*Crataegus sp*) - leaves and berries; used as an astringent for digestive system, diuretic etc. Principal polyphenolic metabolite - proanthocyanidins.

5. **Meadow sweet** (*Filipendula ulnaria*) - aerial parts of the plants, leaves and flowers used as an infusion; antirheumatic, antiinflammatory agent. Principal polyphenolic metabolite - ellagitannins.
The plant polyphenols are distinguished by the following general features.

(a) Water solubility:- In the natural state polyphenol interactions usually ensure some minimal solubility in aqueous media.

(b) Molecular weights:- Natural polyphenols encompass a substantial molecular weight range from 500 to 3000-4000.

(c) Structure and polyphenolic character:- Polyphenols, per 1000 relative molecular mass, possess some 12-16 phenolic groups and 5-7 aromatic rings.

(d) Intermolecular complexation:- In addition to giving usual phenolic reactions, they have the ability to precipitate some alkaloids, gelatin and other proteins from solution. These complexation reactions are of intrinsic scientific interest as studies in molecular recognition and as the basis of possible biological function.

(e) Biological and Chemical properties.

In addition to the general features plant polyphenols show various biological and chemical properties. Invitro testing has identified a wide range of potentially significant biological activities which are exhibited by natural polyphenols and a selection of these is shown below:

(i) Bactericidal action

(ii) Antihelminthic action

(iii) Inhibition of human simplex virus (HSV)

(iv) Antihepatoxic action.

(v) Host mediated antitumour activity
Although these studies have revealed important differences in pharmacological activity between individual polyphenols and between classes of different polyphenols, overall they suggest some selectivity rather than high specificity towards particular biological targets. Thus Okuda (1993) and his collaborators (Kashiwada, et. al, 1992) demonstrated significant inhibition of both the cytopathic effect of HIV and expression of HIV antigen in human lymphotropic virus type I (HTLV-1) - positive MT-4 cells by several hydrolysable tannins. Nevertheless, it is also clear that polyphenols have a number of physical and chemical properties - associated principally with the possession of a concatenation of phenolic nuclei within the molecule - in common. These properties, moreover, probably underly, at least in part their physiological and pharmacological activity.

Natural polyphenols can form complexes with metals and metal ions such as iron, vanadium, manganese, aluminium, calcium etc. Iron is common to all life and the most abundant transition metal. There seems therefore a very good reason to think that natural polyphenols have the potential to modulate physiological reactions involving iron and other transition metals.

According to Halliwell (1995), plant phenols and polyphenols are possibly important as antioxidants and are known to lipid peroxidation and lipoxygenases in vitro, and information has been accumulated over the past few years demonstrating their ability to scavenge radicals such as hydroxyl, superoxide and peroxyl, which are known to be important in cellular peroxidant states.

Although the uses of polyphenols as medicinal agents are earlier seen, their action appear due to their ability to complex with proteins and polysaccarides. They thus
aid the healing of wounds, burns and inflammations. In doing so, they act to produce an impervious layer under which the natural healing can occur. This property to bind to proteins also, presumably accounts for the fact that polyphenols inhibit virtually every enzyme that are tested with *in vitro* (Loomis, 1974). In an earlier study of plant viral infection, Cadman (1960), suggested that polyphenolic extract of the leaf of raspberry probably act on most viruses by clumping the virus particles together into complexes which are largely uninfected. In later works, others have similarly deduced that viral inactivation, in vitro is directly attributable to preferential binding of the polyphenol to the protein coat of the virus (Konishi and Hotta, 1979). In a systematic study of the antiviral activity of a very wide range of natural products, Vaden Berghe et al., (1985) concluded that polyphenols act principally by binding to the virus or the protein of the host cell membrane and thus arrest adsorption of the virus. The complexation of polyphenols with proteins is a specific example of the phenomenon of the molecular recognition

\[
\text{Protein.} \ [\text{H}_2\text{O}]_n + \text{Polyphenol.} \ [\text{H}_2\text{O}]_n \leftrightarrow \begin{bmatrix} \text{Protein.} \\ \text{Polyphenol} \end{bmatrix} \ [\text{H}_2\text{O}]_{m+n}
\]

**Polyphenol-protein association**

Proteins which are small and compact with a tightly folded secondary and tertiary structure have a poor affinity for polyphenols. Conversely, proline-rich proteins (e.g, gelatin, salivary proteins) which have an open, random coil type of confirmation have a high affinity for polyphenolic substrates.

The role of water and water solubility is one of the key factors in the phenomenon of polyphenol complexation. It is observed that where a polyphenol molecule is
highly water soluble, then there will be very little driving force towards complexation with proteins.

Hydrogen bonding is the second effect which dominates polyphenol complexation, and it is thought to be of importance because of its directionality and the overall strength derived from a multiplicity of hydrogen bonds radiating from the polyphenolic substrate. The carbonyl function in tertiary amides (such as polypeptides and caffeine) is a much more hydrogen bond acceptor than that in primary and secondary amides (Cheek and Lilley, 1988).

![Reinforced Hydrogen bonding of a phenolic group with the tertiary carbonyl group of a prolyl peptide.](image)

The reason for this improved binding has been suggested to be the poorer solvation of the tertiary amide function. The interaction of plant polyphenols with the proline rich protein collagen in skins forms the basis for the production of leather.

1.4 Polyphenol in coconut husk and its liberation during retting.

Coconut husk which represent the entire fibrous material enveloping the fruit of *Cocus nucifera* Linn. constituting both the exocarp and the mesocarp, is the raw material for the coir industry from where coir fibre is extracted out. The elastic cellular cork like material forming the non-fibrous tissue of the husk is referred to
as the coconut pith and accounts for as much as 50-70% of the total weight of the
husk; the remainder constitutes pectic substances, polyphenols etc (Bhat, 1969.
1000 husk yield about 82 kg coir. The age of the mesocarp is an important factor in
the quality of fibre and so husks from near ripe to ripe nuts are reported to give best
fibres.

The intense cultivation of the coconut tree is the reason for concentration of the coir
industry along the coastal belt of Kerala.

Preliminary studies on the polyphenols that are present in coconut husk as stated
earlier reveals tannic acid, protocatechuic acid, catechol, pyrogallol etc as the
polyphenols.

The traditional method of natural retting practiced in India consists of soaking the
husks in saline back waters. There are two methods of soaking the husk. The first
method is to arrange the husks in coir nets into bundles and float them freely in the
backwaters until they get soaked, become heavy and gradually sink to the bottom.
The second method practiced is to steep the husk into pits dug within the reach of
tidal action of the backwaters. Whatever be the practice, the principle involved is to
steep the husk for retting in sea water or brackish water. Interestingly unlike other
plant fibres which get released within a few days of retting, the coir fibre takes a
longtime to get separated from the binding material, the retting time varying from 4
to 12 months, depending on the area and the variety of yarns required to be pro-
duced. In fact, the quality of the product is judged by the area from which it is
derived. According to a survey report (survey reports on Coir Industry, 1968), ma-
majority of the retters (67%) ret their husk for a period ranging from 7 to 9 months,
25% ret their husk for more than 9 months and the remainder (8%) do so for 2-6 months.

The principal change brought about in the plant tissue during retting is the breakdown of pectic substances which form the chief constituent of the middle lamellae between the fibre cells and the cementing material. The biological retting of coconut husk differs from that of the other fibrous materials in that it is not confined to pectin decomposition alone but also extents to the disintegration of the phenolic cement binding the fibres together. One of the important observations made in the retting of husk was that polyphenols from the husks get constantly leached out into the surroundings (Jayashankar, 1966). The relatively high percentage of such polyphenols in coconut husk (Varrier and Moudgill, 1947) has been pointed out by Menon & Pandalai (1958) as the very reason for the delay (4-12 months) in the completion of the retting process.

A proximate analysis of the husks best suited for retting and production of good fibre showed polyphenols and pectins to be the more important constituents as they represented 75-76 and 16-17g/Kg of husk materials (Jayashankar, 1966 and Bhat, 1969). For determining the end point of retting, it was considered essential to estimate residual polyphenols and pectins in the retting husks. Experimental results indicated positive correlation between the progress of the extent of retting and the rate of disappearance of these compounds. The gradual fall in the high polyphenol content of the husks during the progress of retting led to ascertaining the nature of polyphenols present in the husk and the type of microflora associated with the process. As indicated earlier it is also assumed that the simple phenols may be found dispersed admist the built in units of plant polyphenols. The colour reactions with
alum, ferrous sulphate, bromine water and p-dimethylamino-benzaldehyde, the polyphenols of the husk were identified as catechin-like tannins (Jayashankar, 1966).

The various factors affecting the retting of coconut husk are the salinity of water (Pandalai et al., 1957), periodic flushing of the ret liquor, which is correlated to the periodic tidal waves which helps in leaching out the polyphenols, crushing of the husk prior to retting for removing the polyphenols to reduce the retting period.

1.5 Environmental effects of polyphenols from coconut husk during retting

One of the striking features of Kerala state is the continuous chains of lagoons or backwaters existing along the coastal region. The backwaters support rich and diverse life forms and provide crucial nurseries for shrimps and fishes as well as habitat for oysters, clams and mussels which later enrich the ocean and make Kerala the principal exporter of marine products amongst the states of India. The shallow fringes of backwaters and the channels drawn from them are used for retting of coconut husk. Retting is brought about by the pectinolytic activity of microorganisms especially, bacteria, fungi and yeasts degrading the fibre binding material of the husks and liberating large quantities of organics and chemicals into the environment including, pectin, pentosans and polyphenols. Coir retting thus becomes a source of pollution to the backwaters of Kerala. From various experiments conducted for investigating and ascertaining the nature of pollution, it is found that there is no variation in temperature between the retting and non-retting areas, the population of fish in the retting areas is negligible when compared to non-retting areas due to the depletion of dissolved oxygen, the pH in the retting areas is between 4.2 to 5.6 as when compared to 7.1 to 7.8 of the non-retting areas. The
contents of phosphates, nitrates and hydrogen sulphides increase in the retting areas compared to the non-retting areas. According to a survey by Remani et al. (1989) the fish population in the retting areas were 20Kg/hectare/month and 60.6Kg/hectare/month in the non-retting areas respectively. The benthic fauna scarcely exist in the retting zones due to the effect of polyphenols released from the husk.

In addition to the disturbances on the flora and fauna in the aquatic system, the polyphenols released during retting also causes skin diseases and irritations on the people who work in the retting yards. This irritation is due to the acidic nature of the polyphenols that are released from the husk in the natural environment.

1.6 Development of bioreactor for retting coconut husk as an alternative method of retting in open waters.

The natural method of retting of coconut husk for extracting out fibre takes long period of 6-12 months. During the initial period much of the polyphenols from the husk get liberated into the aquatic system, this inhibiting the microbial process of retting and eliminating the flora and fauna from the retting grounds. (Menon and Pandalai, 1958). In order to combat pollution and to have total control over the retting process a bio-reactor has been developed as an alternative method (Anon, 1998). In this bioreactor the ret iquor generated during retting is microbially treated and circulated. Since polyphenols are the inhibitory compounds delaying the retting process they have to be removed faster prior to retting and for which a pre-retting operation has to be incorporated. Thus the fabrication of a bio-reactor as an alternative method for coir-retting has the advantage of extracting out polyphenols prior to retting and thus eliminating any environmental impairment due to the process.
1.7 Relevance of this study and its possible impact on coir industry and in the protection of environment.

The present study focuses attention on developing a viable technology which can be designated as the ‘pre retting operation’ which mainly includes stripping polyphenols prior to retting the husk in the reactor. Once the polyphenols are separated they have to be concentrated and the ret liquor thus generated can find various industrial applications. The polyphenols thus form a by-product of coir retting in the bioreactor and to make them a product of commercial importance their physical, chemical and biological characteristics have to be worked out, besides their possible applications.

The study was carried out along with the development of coir retting bioreactor and thus was made an integral part of the technology. So far the coir pith generated during the fibre extraction was the only by-product which has already found its commercial relevance at various fronts. Like wise by upgradating retting process from a natural to the bioreactor mode it is now made possible to recover one more by-product which otherwise would have been lost in the open waters. A proper utilization of the ret liquor and the polyphenols contained in it adds to the revenue and partially bears the cost of running the bioreactor.

In the environmental perspective the present study has great relevance as it totally eliminate the possibility of any environmental impairment due to retting. Through the pre-retting operation the polyphenols are now extractable in water and can be concentrated and can find various bio-medical and industrial applications. This forms the first steps towards the development of a clean technology in coir retting which forms the backbone of the coir industry. As is well known that a ‘clean technology’
is well acceptable than an ‘cleaning up technology’ it is envisaged that the present work shall dramatically transform the coir industry from a traditional, polluting to a modern, environment friendly industry which attracts skilled labour and earns copious foreign exchange.

The work carried out with this as the focus is presented under three chapters such as a). The pre-retting operation b). Characteristics of ret liquor and c). Possible applications with a Conclusion and Bibliography.