CHAPTER 4

DISCUSSION

4 DISCUSSION

The present study is an attempt to evaluate biochemical and pharmacognostic details of South Indian representatives of the family Phytolaccaceae, *viz., Phytolacca octandra* L., *Petiveria alliacea* L. and *Rivina humilis* L. Qualitative phytochemical tests and quantitative estimations of the plant extracts showed the presence of different phytocompounds. The generic composition of the family Phytolaccaceae has been controversial. A synoptic review of past taxonomic treatments revealed a variable assortment of familial circumscriptions and intra familial classifications (Nowicke 1969; Bedell 1980).

4.1 BIOCHEMICAL ANALYSIS

The amount of proteins, carbohydrates, amino acids etc. are crucial as they represent the physicochemical parameters of the drug. The present analysis revealed that there is a slight variation in the amount of protein, carbohydrate, pigments and amino acids among the three genera studied. In many cases, the active principles have been identified as derivatives of carbohydrates and glycoproteins. Polysaccharide protein and protein bound polysaccharide complexes extracted from higher plants and fungi are examples.

From the nutritional point of view, proteins, carbohydrates, amino acids and other such contents are very important. The present study reveals that protein content in the 3 plants studied vary among themselves. Variation in the protein content of different genus and species of plants in a family is common.

It is reported that the young leaves of *Phytolacca americana* L. is used as vegetable. Besides the quantity, the quality of protein is also an important criterion from the nutritional point of view. Proteins present in various edibles differ in their nutritive value on account of difference in amino acid composition. The essential amino acids (EAA) which can not be synthesised in human body have to be supplied through diet. The extent of the presence of these amino acids largely determines the quality of proteins. Approximately 25-30% of the total amino acids occur as free amino acids limiting a range of 10-15%. Out of the eight very essential amino acids, only five are likely to limit the protein quality of mixed human diets, such as lysine, methionine, cysteine, threonine and tryptophan. Arginine and histidine are considered to be essential for children. Histidine is also found to be essential for patients with chronic liver diseases (Robert *et al*. 1993). The present analysis reveals that the percentage of free amino acids in *Petiveria alliacea* L. and *Phytolacca octandra* L. are higher than that of *Rivina humilis* L..

The amino acids were separated and quantified by HPLC (High performance liquid chromatography) and it was observed that the essential amino acid Methionine was present in highest percentage in *Phytolacca octandra* L. The amino acid present in minimum quantity in *Petiveria alliacea* L. was aspartic acid and that in *Rivina humilis* L. was glycine. But the essential amino acid cysteine was found to be absent in all the three plants studied. The percentage of various amino acids reveals that the 3 plants studied are rich source of amino acids both essential and non essential.

Chlorophylls are a group of natural porphyrins containing a chelated magnesium atom in the centre and a long diterpenoid phytol chain attached through a carboxylic acid group. Higher plants contain chlorophyll *a* and chlorophyll *b*. Chlorophylls occur along with carotenoids in plants.

From the analysis it was clear that the pigments of *Phytolacca octandra* L. and *Petiveria alliacea* L. are similar but slightly differ from *Rivina humilis* L. Earlier works suggest that all of the chlorophyll *b* and xanthophylls of higher plants and green algae are not present in pigment system II but occur in another pigment protein complex known as light harvesting chlorophyll protein complex (LHCP complex) (Goodwin and Mercer 1986). The existence of this could also be inferred from the analysis of the ratio of chlorophyll *a* to chlorophyll *b*. Pigment estimation followed by the computation of the chlorophyll ratio showed variation in the levels of

LHCP. The increase in the amount of chlorophyll *b* or decrease in the chlorophyll *a/b* ratio indicated the development of LHCP. Chloroplasts with higher chlorophyll *a/b* ratio have less LHCP and more of their chlorophyll is associated with the reaction centre complex than chloroplasts with lower chlorophyll *a/b* ratios (Anderson, 1980). This suggests that at low light intensity the antennae pigments of PSI and PSII are incapable of absorbing enough light photons to maintain the photosynthetic rate constant. In the present observation *Phytolacca octandra* L. showed high photosynthetic efficiency compared to the other two genera studied. This was also reflected in the percentage of carbohydrate synthesised. Among the three plants *Phytolacca octandra* L. had high percentage of carbohydrates and *Petiveria alliacea* L. had minimum percentage of carbohydrates. *Rivina humilis* L. came next to *Phytolacca octandra* L. in the amount of carbohydrate produced.

Starch, the storage polysaccharide of higher plants, consists of two components *viz.,* amylose and amylopectin. Amylose component consists of D-glucose units linked linearly by α-1-4 glycosidic linkage. It has a non reducing end and a reducing end. Amylopectin is a branched polysaccharide of glucose units linked by α-1-4 linkage and α-1-6 linkage (Sadasivam and Manikkam, 1992; Jayaraman, 1981). Much has been learned about the structure of starch by the action of enzymes on polysaccharides. β-amylase, an enzyme found in plants, attacks the non reducing end of amylase to yield

successive units of maltose, which can be hydrolysed to the reducing sugar α-D-glucose. Amylopectin hydrolysed by amylase enzyme yield highly branched short chains called limit dextrins (Lehninger 1991; Conn and Stumpf 1990).

The specific activity of the enzyme amylase was studied in germinating seeds. The activity of amylase enzyme is an indication of the presence of starch as reserve food material in seeds. The specific activity of the enzyme was almost similar in the three genera studied.

Even though there was variation in the amount of protein, the polypeptides analysed by SDS-PAGE showed more or less same profile in all the three plants. A prominence of low molecular weight polypeptides was observed in the plants under study. The presence of polypeptides of molecular weight 33kD and 28kD indicated the presence of extrinsic proteins associated with PSII and LHCP complexes. Presence of polypeptides of molecular weight above 60kD represents either the structural protein or enzymes capable of inducing growth hormones.

4.2 PHARMACOGNOSTIC ANALYSIS

The universal role of plants in the treatment of disease is exemplified by their employment in all the major systems of medicine irrespective of the underlying philosophical premise. The word Pharmacognosy is derived

from the Greek words 'Pharmakon', which means "a drug" and gignosco, means "to acquire knowledge of" (Evans 2002). Qualitative phytochemical tests and quantitative estimations of the active extracts demonstrated the presence of phyto-compounds in the plant extracts such as phenolics, alkaloids, tannins, resins, saponins, flavonoids and steroids as the major active constituents.

A serious limitation encountered in the use and research of traditional medicine is the lack of standardization and quality control of raw materials forming the drug. The ultimate objective of pharmacognostic investigation is the identification of the genuine crude drug and determination of extent of adulteration or substitution if any. Advancement in pharmacognosy, phytochemistry and physicochemical instrumentation techniques is of immense value in rectifying this major shortcoming of traditional medicine. These techniques can be utilized for correct botanical identification of plants. There are about 35,000 plant species being used in various human cultures for medicinal purpose throughout the world. It is estimated that the folk medicine including tribal medicine in Kerala uses about 500 single plant remedies and more than 200 sample formulations.

The active molecules isolated from traditional medicinal plants might not only provide valuable drugs but are also valuable as 'lead molecules' which might be modified chemically or serve as a template for the design of synthetic molecules.

The present investigation was aimed to evaluate the South Indian representatives of the family Phytolaccaceae. Macro and microscopic characters, behaviour of the powdered 'drug' on treatment with different chemical reagents and preliminary phytochemical and pharmacognostic tests were carried out to study the distinctive features of the plant. Such parameters provided basis for standardization and characterization of a genuine drug.

The leaf architecture and stomata of different angiosperms have been studied by several researchers because of the significance of these structures in taxonomy. Metcalfe and Chalk (1950) classified the stomata as anomocytic, anisocytic, diacytic and paracytic and studied the structure and ontogeny of stomata in different plants. A number of leaf measurements were used to distinguish between some closely related species not easily characterized by general microscopy (Evans 2002).

Stomatal index is highly constant for a given species and can be determined on either entire or powdered samples (Evans 2002). The stomatal index and palisade ratio are species specific characters and therefore much counted in pharmacognostic studies (Wallis and Forsdike 1938). In the present investigation the *Phytolacca octandra* L. and *Rivina humilis* L. showed almost similar index values for stomata. But the stomatal index of *Petiveria alliacea* L. was higher than the other two. The vein islet

number and veinlet termination number of *Phytolacca octandra* L. and *Petiveria alliacea* L. showed more similarity than *Rivina humilis* L.

The variations in the major venation patterns are significant for phylogenetic considerations. The classification of venation pattern was studied by Hickey (1973) and discussed the taxonomic significance of vein - islets and vein endings. Of all plant organs leaves are highly polymorphic and provide sets of diverse features. Moreover, there are many taxa with similar leaf forms and therefore it seems rather difficult for plant taxonomists to rely upon this character. The veins and vein lets which form the vasculature called the venation is an important feature of both mature leaves and cotyledons. The absolute vein islet numbers of some Solanaceous plants were studied. Hickey (1973), and Melville (1976) studied and classified the architecture of leaves of angiosperms.

The phylogenetic significance of venation in angiosperms was reported by Foster (1961). Veinlet termination number was reported as a character for differentiation of species by Melville (1976). Since the leaf architecture and venation are specific for groups or species, the difference shown by *Rivina humilis* L. is significant on the taxonomic point of view. The evolutionary significance of leaf architecture was emphasised by earlier researchers (Foster 1968; Hickey 1971; Varghese 1998). The microscopic features of plant powder studied revealed that there were only slight variation among the characters observed in the 3 plants.

The qualitative analysis of phytoconstituents confirmed the presence of alkaloid, flavonoid, resin, tannin, saponin and bitters. Volatile oil was absent in all the 3 plants studied. The fluorescence analysis showed more similarity between *Phytolacca octandra* L. and *Rivina humilis* L. than *Petiveria alliacea* L.

It was found that saponins were absent in *Rivina humilis* L. but *Phytolacca octandra* L. was observed as a rich source of saponins. The HPTLC analysis showed the presence of Benzoic acid in the 3 plants. But gallic acid and sterol were reported in *Petiveria alliacea* L. and *Rivina humilis* L. They were absent in *Phytolacca octandra* L. The studies revealed that the three plants studied are rich source of phytoconstituents. It was reported that the berries of *Phytolacca dodecandra* L. synthesize various triterpenoid saponins, which possess potent and useful biological properties including detergent, molluscicidal (Lemma 1965; Parkhurst *et al.* 1974; Lemma *et al.* 1991), spermicidal (Stolzenberg and Parkhurst 1976) insecticidal (Spielman and Lemma, 1973) and fungicidal actions. Most scientifically studied use of *Phytolacca dodecandra* L. is its molluscicidal property. It is referred to as the biodegradable plant molluscicide (Lambert *et al*. 1991; Molgaard *et al*. 2000).

In the present analysis it is observed that the plants studied have high microbicidal activities. The bioactivity analysis of the plant extract showed antibacterial and antifungal properties against all the tested pathogenic

organisms. The results indicate that the different extracts have variation in inhibitory potencies due to the differences in the phytoconstituents of the extracts. The three plants studied have antimicrobial activity, which is as potent as standard antimicrobial drugs against the microorganisms. The microorganisms studied are industrially important pathogens.

4.3 PHENOTYPIC VARIATION AND GENETIC DIVERSITY AMONG ACCESSIONS

As per the correlation and ANOVA results of the samples morphological ecotypes are not present in the sample sites. The earlier studies on phenotypic variations in *Phytolacca dodecandra* L. also were not supported the presence of morphological ecotypes along altitudinal gradients (Semagn *et al*. 2004).

Random Amplified Polymorphic DNA (RAPD) analysis is a genetic assay technique to detect genetic differences (Polymorphism) in the DNA from different individuals. When DNA from different genotypes are subjected to RAPD assay using the same primer, different DNA segments may or may not be amplified, depending on the nucleotide sequence of the template. The resulting polymorphism is due to genetic differences. Polymorphism was detected by the presence or absence of an amplification product from a single locus.

RAPDs are scored as discrete variables using '1' to indicate the presence and '0' the absence of bands. Only those fragments, which are repeatable and clearly scorable across all genotypes shall be considered for interpretations of genetic differences. Using this data genetic distance among genotypes can be estimated. The genetic distance matrix is used in cluster analysis, by statistical method like UPGMA. This facilitates grouping the genotypes into clusters based on their genetic relationships (Annamma 1998).

RAPD analysis was reported as quick cost effective techniques for preliminary screening, quantification and partitioning of molecular variation among accessions (Phipper *et al. 1997).* RAPDs have been extensively used for identification of genetic differences in cultivated germplasm. The use of RAPD to determine genetic relationships have been demonstrated in a large number of species like maize (Welsh *et al.* 1991); rapeseed (Forster and Knaak 1995), Indian mustard (Jain *et al.* 1994), faba bean (Link *et al.* 1995) and many other species. Similarly RAPD markers have been used for cultivar identification in celery (Yang and Quiros 1993), broccoli and cauliflower (Hu and Quiros 1991) grape vine (Moreno *et al.* 1995). Unique fingerprints of a number of grape vine cultivars were readily distinguished by using either a single primer or a mixture of two.

Tinker *et al.* (1993) in a study on RAPDs and pedigree relationships among spring barley lines with varying amounts of common ancestry,

concluded that RAPD markers can be used to gain information about genetic similarities or difference that are not evident from pedigree information. Similar was the observation from 24 rather closely related clones of *Hevea brasiliensis* M. Arg. (Varghese *et al.* 1997).

Useful polymorphisms have been found at a variety of taxonomic levels ranging from varieties and cultivars to species and above species level (Hedrick 1992; Ellsworth *et al.* 1993; Smith *et al.* 1994). Though the RAPD analyses in Phytolaccaceae are limited, it was employed for assessing the genetic diversity and relationships in many plants. RAPD analysis was used as a tool to identify *Cajanas cajan* (L.) Millsp. cultivars and their wild relatives by Ratnaparkhe *et al.* (1995). The differences in morphology and the geographical origin of genotypes were reflected in the RAPD patterns of *Coffea arabica* L. (Orozco *et al.* 1994). The genetic relationships between members of the family Fagaceae was assessed by RAPD techniques (Gallois *et al.* 1998). Rajasegar *et al.* (1997) have also demonstrated the usefulness of RAPD analysis in cultivar development in *Ixora,* a group of tropical ornamental plants. The potential use of RAPD technique to study the genetic diversity in *Brassica juncea* Hk.f&T. and its relationship to heterosis was successfully demonstrated by Jain *et al.* (1994). RAPDs help in improving the efficiency of breeding programmes in identifying genetic variation among different individuals, cultivars, races, species or genus (Keil and Griffin 1994; Varghese 1992). A good number of examples of

association of RAPD markers with desired crop species are available (Chague *et al.* 1997; Hu *et al.* 1997; Page *et al.* 1997; Naqvi *et al.* 1995; Zhang *et al*. 1994; Eastwood *et al.* 1994; Oh *et al*. 1994; Williamson *et al*. 1994; Salentijin *et al*. 1995; Wilde *et al.* 1992; Lashiermes *et al*. 1993).

Semagn, *et al.* (2000 and 2001) used RAPD technique to investigate the genetic diversity and structure of *Phytolacca dodecandra* L. population. The study demonstrated the presence of clear genetic differentiation of the populations into two or three altitude groups. The molecular data supports the presence of different ecotypes and are different in ploidy level.

In the present study numerical treatment of the RAPD data obtained from the accession of the 3 plant samples was performed according to UPGMA method which showed 3 major clusters. Cluster I comprised *Petiveria alliacea* L. from Ernakulam, Trivandrum and *Phytolacca octandra* L. from Kodaikanal. *Rivina humilis* L. from Ernakulam, Trivandrum and Muvattupuzha formed the cluster II. The cluster III was comprised of *Phytolacca octandra* L. from Munnar and Ootty.

It is hypothesized that the different clusters may be different in physiology or chemistry or the differences may be as a result of complex interactions of climatic or edaphic factors. They may be different in ploidy levels or the groups may be different ecotypes.

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