CHAPTER 1

INTRODUCTION

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1 INTRODUCTION

We share our planet with millions of species of different plants and animals. In order to communicate, retrieve, store and accumulate information about our co-inhabitants, it has been necessary to identify the organisms, name them and to place them into groups that reflect their evolutionary relationships. With the huge variety of plants surrounding us, it is extremely essential to pinpoint a particular plant of our interest by analysing the similarities or difference with other plants.

Knowledge of the plant chemistry is very much essential for the development of useful plant products. It improves the understanding and utilization of various economic products. Chemical constituents of organisms are taxonomically valuable and have the advantage over classical characters that they can be exactly described. The purposeful search for certain useful compounds in related taxa has been of great interest to the pharmaceutical and food industry.

Plants are the exclusive source of drugs for majority of the world population even today. In fact in the plant kingdom, there are thousands of plants both known and unknown that have the potential to yield drugs of great use to man but is still unexplored. The subject of Modern Pharmacognosy is

one of the youngest of all medicinal sciences. The world's oldest pharmacological and therapeutic writings came from India and China. Pharmacognosy also is vastly updated by the incorporation of phytochemical methods. Modern aspects of pharmacognosy include not only the crude drugs but also their natural derivatives. A drug is studied with respect to its constituents, active principles, their isolation, identification and evaluation. This was done by conventional methods earlier, but now it is supplemented by chemical analytical techniques (Daniel 1991).

The earliest Indian records are the Vedas. Although, there are medical description in Rigveda (2500 B.C – 3000 B.C), it was Charaka, a renowned ancient Indian Physician and later Sushruta and Vagbhatta who described the various medicinal preparations included in Ayurveda.

Tropical and sub-tropical countries have plenty of medicinal herbs. Herbs have a special role in village health care. Use of local medicinal plants saves a lot of time and money spent on health care. Herbal medicine is still the mainstay of about 70-80% of the world population, mainly in the developing countries, for primary health care because of better cultural acceptability, better compatibility with human body and lesser side effects. The documents, many of which are of great antiquity, revealed that plants were used medicinally in China, India, Egypt and Greece long before the beginning of the Christian era. Ayurveda the ancient science of Life – is

believed to be prevalent in India for the last 5000 years. It is one of the most noted systems of medicine in the world.

The expanding knowledge of phytochemical screening has revealed the existence of close relationship between constituents of plants and their taxonomical status. The characters more often studied in chemotaxonomy are secondary metabolites of pharmaceutical significance such as alkaloids, glycosides, flavonoids etc. (Kokate, *et al.* 2004).

India is a mine of well recorded and well practiced knowledge of traditional herbal medicine. But unlike China, India has not been able to capitalize on this herbal wealth and tap the hidden potential of the rich biodiversity (Handa and Kapoor 1999). The unique diversity of natural vegetation of India owes to the wide range of soils and agro climatic conditions. The fundamental basis of Indian medicine is plant drugs alone. It harbours 17,500 flowering plants out of which only 1500 species are used in various systems of medicines like 'Ayurveda', 'Siddha' and 'Unani'. South India especially Kerala, Andhra Pradesh and Karnataka are homes of number of commercially important medicinal plants (Kamboj 2000).

1.1 REVIEW OF LITERATURE

The floristic composition of India and of any other country, tends to change in course of time due to extinction, evolution of new species,

immigration and introduction. Many of the weeds found here, are reported to have important medicinal values in their native homes. One such example is the members of the family Phytolaccaceae (Sivarajan and Indu 1987). The family Phytolaccaceae is a family in dispute. Different authors classified the members of the family in different methods and placed under different orders. The name *'Phytolaccaceae'* is because of the presence of a special dye [*Phytol* (Greek) means plant and *lac* (French) means a dark pigment)]. Members of this family are quite interesting, because they are medicinal as well as toxic to human. *Phytolacca americana* L. is referred to as an interesting American vegetable. It is a delicious vegetable if the toxic content is removed by boiling.

Phytolaccaceae is commonly known as Pokeweed family. The name Phytolaccaceae Lindl. is conserved over Petiveriaceae Link. (Lawrence 1974). Three genera of the family Phytolaccaceae are indigenous to South India; the Pokeberry (*Phytolacca)* from Maine to Minnesota and southward, the Pigeon berry (*Rivina*) from Florida to Texas and Garlic weed, a monotypic genus (*Petiveria)* whose northern limits are in Southern Florida (Rendle 1956).

Species of *Phytolacca* and *Rivina humilis* L. are grown for ornamental purpose. The species of *Agdestis, Ercilla* and *Petiveria* are also ornamental. Young shoots of *Phytolacca* and *Rivina* are source of *edible greens* (Lawrence 1974; Hooker 1983). The fruits of *Phytolacca decandra* L, the

deep purple berries, yield a deep red dye which is used for colouring wines etc. (Rendle 1956).

Different aspects of the family Phytolaccaceae were studied by many workers. The morphologic, taxonomic and anatomical features were analysed by different workers such as Walter (1909), Lloyd (1917), Salisbury (1927), Toole and Brown (1946), Sauer (1950), Hall and Melville (1954), Steinmetz (1960), Dutta and Mitra (1961), Balfour (1965), Byrd (1966), Willis (1966), Fahn and Shchori (1967), Cronquist (1968), Nowicke (1969), Matthew (1983), Nowicke (1969), Krochmal (1970), Hutchinson (1973), Behnke *et al.*(1974), Inamdar and Patel (1976), Wheat (1977), Mikesell (1979), Bedell (1980), Mikesell and Allen (1980) Cronquist (1981), Horak (1981), Armesto *et al.* (1983), Ghosh and Sikdar (1983), Brown and Varadarajan (1985), Rogers (1985), Sivarajan and Indu (1987), Jan (1988), Julieta and McDonald (1989), Margaret and Susan (1990), Cronquist and Throne (1994), Dhruvan *et al.* (1997), Steven *et al.*(2000). Austin (2001), Werner (2001) and Mats (2003).

1.1.1 CLASSIFICATIONS OF PHYTOLACCACEAE

Different taxonomists classified Phytolaccaceae on different basis. The familial relationship and phylogenic aspects were analysed by Sauer (1952), Hershkovitz (1989), Giannasi *et al.* (1992), Rettig *et al.* (1992), Rodman (1994), and Park (1995). According to Bentham and Hooker (1979)

the family is divided into three tribes. The 19 genera were distributed among those three tribes. Hutchinson (1964 and 1973) placed the members under the order Chenopodiales in five families. The order was comprised of 21 genera under the five families. According to him the family Phytolaccaceae has only three genera such as *Anisomeria*, *Ercilla* and *Phytolacca*. The three genera growing in India, other than *Phytolacca*, namely *Petiveria*, *Trichostigma* and *Rivina* were placed under the family Petiveriaceae. The rest of the members were distributed to the other families like Barbeuiaceae, Gyrostemonaceae and Agdestidaceae.

According to Lawrence (1974) the family Phytolaccaceae consists of 17 genera and 125 species, largely of American tropics and sub tropics. *Phytolacca* is the largest genus of the family with 35 species. Rendle (1956) placed the family Phytolaccaceae under the order Centrospermae as a small family of 17 genera and 115 species comprising herbs, shrubs and trees widely distributed in tropical and temperate climates and found mainly in warm parts of America. Cronquist (1968) placed the family Phytolaccaceae under the order Caryophyllales of the sub class Caryophyllidae.

According to Willis (1973) the family Phytolaccaceae comprises 12 genera and 100 species, being chiefly distributed in tropics and subtropics. Hooker (1983) recorded only one species, *Phytolacca acinosa* Roxb. under the genus *Phytolacca* Linn., from India.

Walter (1909) published a comprehensive treatise on the family Phytolaccaceae consisting of 22 genera and 114 species. He placed the genus *Phytolacca* Linn. in the sub tribe Phytolaccinae H. Walt. of the tribe Phytolaccae Reichb. under the sub family Phytolaccoideae H. Walt. The other three genera such as *Rivina* Linn., *Trichostigma* A. Rich and *Petiveria* Linn., found in India, were grouped in the tribe Rivineae Reichb., under the subfamily Phytolaccoideae H. Walt. Huctchinson (1973) classified Phytolaccaceae into five families such as Phytolaccaceae, Barbeuiaceae, Gyrostemonaceae, Agdestidaceae and Petiveriaceae. According to him, all the Indian genera were belonging to Phytolaccaceae and Petiveriaceae. Dutta and Mitra (1961) recorded a new taxon *Petiveria alliacea* L. from West Bengal and this record has added an element to the Flora of India. *Petiveria alliacea* L. was reported from the peninsular India (Trivandrum) by Dhruvan *et al.* (1997).

1.1.2 GENERAL DESCRIPTIONS OF THE FAMILY

The family Phytolaccaceae includes herbs, shrubs and trees. *Leaves are* alternate, simple, exstipulate, entire, glabrous and pinnately reticulate. *Flowers* - in terminal, axillary or leaf-opposed racemes; bisexual or if unisexual actinomorphic or zygomorphic, apetalous, bracteate and bracteolate. *Perianth* (tepals) are 4-5 partite, simple, membranous, free, imbricate in bud, persistent and white or coloured. *Stamens* - 3 to 10 (the number varying within the same species); usually irregularly inserted on a

fleshy hypogynous disc or alternate with the perianth segments. Filaments are free or rarely connate below and persistent. *Anthers* are 2-locular, dorsi or basifixed and open longitudinally. *Carpels* - 1 to many, free or united; ovary superior, ovule solitary in each carpel, basal; styles as many as carpels, short or none; stigmas linear to filiform. *Fruits* - variable, as many as carpels: free or connate, juicy or dry. *Seeds*- erect to reniform with longer peripheric embryo enclosing the abundant endosperm (Ghosh and Sikdar 1983).

Taxa are mostly inhabitants of the tropics of both hemispheres in America, chiefly South America. A few species are found in Europe, Asia and Africa. In India, the family constitutes 4 genera, all introduced from tropical America (Ghosh and Sikdar 1983).

1.1.3 THE SOUTH INDIAN MEMBERS OF PHYTOLACCACEAE

The family Phytolaccaceae (*sensu lato*) in India had been revised by Ghosh and Sikdar (1983) and it is reported that the family comprises 3 South Indian genera. The South Indian representatives of the family are: (1) ***Phytolacca octandra* L.** (Distribution of the genus *Phytolacca* : Tropical and sub-tropical mostly confined to America with a few species occurring in Africa and Asia, one species extending to Canada and 3 species in India. Several ornamental species are cultivated in Europe and elsewhere.), (2) ***Petiveria alliacea* L.** (Distribution of the genus *Petiveria* : Native of Central America; naturalized and reproducing itself by seeds here and there in the

environment of Bogor (West Java) and in some localities of West Bengal (India). Besides it is distributed in West Indies, Jamaica, Mexico to Brazil and Malayan Archipelago.), and (3) ***Rivina humilis* L.** (Distribution of the genus *Rivina*: Native of tropical and sub-tropical America but introduced in Asia and Africa).

The genus *Petiveria* is named after James Petiver, 1665-1718, an apothecary and Botanist of London. According to Walter (1909) the genus *Petiveria* consists of 2 species. But Santapau and Henry (1972) mentioned that the genus has probably one polymorphic species. Willis (1973) also marked it as polymorphic species. Behnke *et. al.* (1974) also supported the view that *Petiveria* is a monotypic genus and they have detected betalains and P-type sieve – tube plastid in *Petiveria* and supported the assignment of the genus to the Centrospermae.

The genus *Rivina* is named after A.Q. Rivinus, Professor of Botany and Medicine at Leipzig 1691-1725 (Ghosh and Sikdar 1983). According to Walter (1909) the genus is represented by 3 species. Rivina is a native of tropical and sub-tropical America but introduced in Asia and Africa. The genus is represented in India by only *Rivina humilis Linn.* which is grown in Indian gardens as ornamental plant and now naturalized.

PHYTOLACCA OCTANDRA L.

Phytolacca octandra L. is a native of tropical America. It has been introduced long ago to Kodaikanal and Nilgiri Hills in India. Now it seems to be restricted to Kodaikanal, Ootty and Munnar in South India, as a common and abundant wayside weed. It also grows along water-sides, field border, waste places etc. at higher elevations along Western Ghats (Sivarajan and Indu 1987; Mathew 1983). It was also distributed from Mexico to Columbia, Malaysia, North Sumatra, Java, Australia and Africa, elsewhere locally naturalised in many tropical countries. The plant emits a foul odour when bruised. Young sprouts and leaves can be used as a vegetable. Flowering and fruiting occur mostly throughout the year but preferably in late summer, *i.e*., May to August.

Phytolacca octandra L. is a glabrous perennial herb of about 1.5 to 2.0 m height. The stem is stout, fleshy and green at the young stage, but on maturity becomes slightly pinkish. The leaves are winged from the decumbent leaf base. Leaves are long lanceolate or ovate-lanceolate and cuneate at the base. The leaf margins are slightly pinkish when mature. Flowers are in racemose inflorescence. The inflorescence is very dense and cylindrical. Flowers are hermaphrodite and regular with short pedicel. Narrow bracteoles are present. 5 persistent perianth segments are present which are green in colour. Usually 8 stamens are present alternating with the carpels. They are inserted on the outer margin of the disc. The filaments are

persistent. Anthers are 2 celled and yellowish. Ovary is sub-globose and greenish. Carpels are laterally connate. One ovule each is present in each carpel. The style is filiform and free. Fruits are globose berries. They are purplish black in colour with black shining small seeds.

The seeds can withstand burial for more than forty years (Toole and Brown 1946) and establish themselves quickly away from the parental plants on disturbance. This ability might have contributed substantially for the dissemination and establishment of the genus.

PETIVERIA ALLIACEA L.

Petiveria alliacea L. is indigenous to the Amazon rainforest and is also found in other areas of tropical America, Africa and Caribbea, naturalized and reproducing itself by seeds, where it is propagating spontaneously but slowly. It is also distributed in West Indies, Jamaica, Mexico, Brazil, Malayan Archipelago and India.

It was first reported in India, to have got naturalised in West Bengal (Dutta and Mitra 1961; Ghosh and Sikdar 1983). In India it is now growing as wild only in some areas of West Bengal and South Kerala. It is growing in waste places in the gardens and village surroundings along the road-sides, especially in shady places.

Petiveria alliacea L. are erect herbs of about 1.0 to 1.5 m height. Stems are shrubby at the base. Leaves are obovate. Minute stipules are

present. Flowers are arranged in racemose inflorescence. The inflorescence is terminal or axillary in position, often nodding at the apex. Bracteoles and 4 persistent perianth segments are present. 8 stamens are present, of which 4 are long and 4 are short. Anthers are two celled. A one carpelled semi globose superior ovary with hook like bristles at the tip is present. Stigma is filamentous and style is absent. Fruits are elongate and cuneate. They are covered at the base by 4 persistent perianth segments. Two curved spines are present at the tip of the fruit. Seeds are solitary, erect and linear. The fruits often adhere to the clothes of passers by. The flowering and fruiting season is from October to February. The species has an odour that is very similar but different to garlic. The plant is also called garlic weed.

RIVINA HUMILIS L.

Rivina humilis L. is indigenous to United States of America., distributed from Florida to Texas. It is now naturalized in Madagascar, tropical South East Asia, Malaysia, Indonesia, Philippines, Singapore, Bangladesh, West Bengal and other parts of India, Pakistan, Burma, Sri Lanka, Eastern Asia and West Indies.

In India it is distributed in West Bengal, Bihar, Meghalaya, Uttar Pradesh, Karnataka, Rajasthan, Maharashtra, Tamil Nadu and Kerala. The species is growing in waste places, hedges, open jungles, and gardens, preferably in shady localities.

Rivina humilis L. is an erect perennial herb with sulcate branches which are woody at the base. Leaves are glabrous and obovate. Leaf base is rounded and truncate. Flowers are hermaphrodite and arranged in axillary or terminal racemes. Flowers are pedicellate and bracteolate. Flowers are white suffused with pink. 8 stamens are present. The anthers are 2 celled. Flowers are having a single carpel with sub-globose ovary. The style is short with a peltate stigma. Fruit is a bright red berry, globose and indehiscent. Lenticular seeds are present. Flowering and fruiting is from June to November (Ghosh and Sikdar 1983).

1.1.4 BIOCHEMICAL IMPORTANCE

Sivarajan and Indu (1987) discussed the medicinal properties of plants of the family Phytolaccaceae. Species of *Phytolacca* have been found to have some important medicinal uses as well. The tincture of *Phytolacca decandra* L. was reported as a remedy for cancer in America and widely used in treating some form of chronic rheumatic complaints. The roots are reported to be emetic and cathartic. The use of *Phytolacca* as a narcotic and medicine has attracted the attention of phyto-chemists, for the last several decades and a formidable number of bioactive compounds have been detected (Sauer 1950; Steinmetz 1960; Byrd 1966). The triterpenoid saponins isolated from species of this genus are collectively called 'phytolacca toxins', which aroused considerable interest as biodegradable, fairly safe molluscicides for controlling schistosomiasis.

Some of the members of Phytolaccaceae are reported as toxic, sometimes even fatal, to human and livestock (Kingsbury 1980). The plants as a whole and leaves and fruits in particular cause gastric irritation, haematological problems, depression of the central nervous system with inhibition of heart and respiration, mental aberration, convulsions and dermatitis on contact (Rogers 1985). Biochemical studies were conducted on many members of the family Phytolaccaceae. Even though the studies on South Indian representatives are less, the other members were studied in detail. Some of the important studies conducted are Steinmetz (1960), Farmer and Hall (1970), Barque and Lequesne (1971), Mabry *et al.* (1972), Waxdal (1974), Woo and Kang (1977), Woo and Wagner (1977), Dallal and Irvin (1978), Suga *et al.* (1978), Woo *et al.* (1978), Irvin *et al.* (1980), Kingsbury (1980), Murray and Thompson (1980), Woo *et al.* (1980), Barbieri *et al.* (1982), Masuho *et al.* (1982), Taylor and Powell (1982), Razdan *et al.* (1982), Woo *et al.* (1982), Houston *et al.* (1983), Dellaporta *et al.* (1983), Forni *et al.* (1983), Woo and Kang (1985), Kang and Woo (1986), Watson and Thompson (1986), Kang and Woo (1987), Doyle and Doyle (1987), Slacanin *et al.* (1988), Kang and Woo (1991), Fang *et al.* (1992), Parente *et al.* (1993), Downie and Palmae (1994), Moon *et al.* (1994), Tanigawa *et al.*(1995), Del *et al.* (1997), Johnson *et al.* (1997), Moon *et al.* (1997), Williams *et al.* (1997), Novruzov (1998), Antimo *et al.* (1999), Park *et al.* (1999), Yingfang *et al.* (2000), Benevides *et al.* (2003), Cho *et al.* (2003), Knight and Walter (2003), Aceto *et al.* (2005), Stanley *et al.* (2005), Antimo *et al.* (2007),

Chambery *et al.* (2007), Di Maro *et al.* (2007), Sriwanthana, *et al.* (2007), Chambery *et al.* (2008), Parente *et al.* (2008) and Webster *et al.* (2008),

The "poke-weed mitogens" (PWM) have the ability to stimulate mitotic proliferation following morphological alternations in the lymphocytes are still being studied. However, young shoots and leaves of some species are eaten by the Himalayan people after boiling and decanting a couple of times to remove the toxins (Hooker 1983; Waxdal 1974). Despite toxicity, the fruits have been used in colouring edibles and beverages (Forni *et. al*. 1983) as a source of ink and as poor dye for fabrics (Rogers 1985). Some species have been cultivated as ornamental plants.

Though little phytochemical, pharmacological and clinical studies have been done on the species *Phytolacca octandra* L., reports on closely related taxa points to the possibility for the presence of similar properties in this species too.

A new glycoside named 'Esculentoside S', a phytolaccagenin, was reported from the leaves of *Phytolacca acinosa* Roxb. (Sigrid and Willi 2008). Phenolic compounds and other elements in leaf extracts were analysed and reported by Yong *et al*. (2005).

Hang and Kai-Mei (2008) examined the allelochemical effects of *Phytolacca esculenta* Van., *Phytolacca insularis* L. and *Phytolacca*

americana L. They have reported that the total phenolics in *Phytolacca americana* L. was two times higher than the other two species analysed.

Analysis of aqueous extracts of *Phytolacca americana* L. showed the presence of seven phenolic compounds namely, gallic acid, protocatechuic acid, chlorogenic acid, caffeic acid, m-hydroxybenzoic acid, coumaric acid and cinnamic acid (Wu 2007).

The phytotoxic effects of the species, *Phytolacca esculenta* Van., *Phytolacca insularis* L. and *Phytolacca americana* L. were different, even though the levels of total phenolic compounds were similar. They can be distinguished by their allelopathic potential and morphologies (Yong *et al.*, 2005; Kim *et al.* 2005).

Strong anti-inflammatory saponins were isolated from callus mass derived from stems and roots of *Phytolacca americana* L. Triterpenoid saponins such as Phytolaccoside A, B and D were also reported (Hung and Hyun 1985).

It is reported that the leaves of *Phytolacca heterotepala* H. Walter., a Mexican pokeweed, contain about ten Type I Ribosome-inactivating proteins (RIP) called heterotepalins. The molecular weight of these heterotepalins were analysed by SDS – PAGE as in the range 28,000 – 36,000. The Type I RIP isoforms were purified by conventional chromatographic techniques and amino acid sequence of *Phytolacca heterotepala* H. Walter. antiviral proteins

were also deduced from c DNA sequence of Ph RIP 1 gene (Antimo *et al.* 2007).

Fujii *et al.* (2004) isolated four types of Pokeweed lectins (PL) from the roots of pokeweed (*Phytolacca americana* L.*).* They were found to contain homologous domains. But the molecular size and biological properties were different. The smaller lectin group, referred as PL-D, had two isolectins, PL-D1 and PL-D2. The PL-Ds were composed of two repetitive chitin binding domains, with four S-S bridges and two carbohydrate-binding sites. The pokeweed lectin is specific for N-acetylglucosamine containing saccharides. It stimulates peripheral lymphocytes to undergo mitosis by binding to their cell surface (Fujii *et al*. 2004).

Knight and Walter (2003) reported that Pokeweed (*Phytolacca americana* L.) causes excessive salivation in animal characterized by drooping or frothy saliva around the lips. All parts of the plant contain saponins, oxalates and the alkaloid phytolaccine with greatest concentrations in the roots and seeds. Pokeberry also contains a mitogen (lectin) which can cause wide effect on the immune system. It is also reported that the species of *Phytolacca* found in South America and Africa have caused higher mortality rates in animals. Humans also poisoned by pokeweed and developed mouth irritation, stomach cramps and vomiting (Peixoto *et al.* 1997; Kingsbury and Hillman 1965; Storie *et al.* 1992).

Phytolacca dodecandra L. (Endod) is a proven botanical pesticide. Because of its larvicidal effects it can be used against larva of mosquito and other insects such as house fly. It has been shown that due to its antifungal property Endod is effective against dermatophytes and is recommended for the preparation of a natural antimycotic ointment. Endod had been found useful in controlling a plague of a bivalve mollusc commonly known as "Zebra mussel" (Lambert *et al.* 1991; Haile 1994). Endod powder when mixed with water forms a detergent solution, that has been traditionally used in Ethiopia for washing clothes. The berries of Endod were also used in fields for killing snails (Lemma 1965).

The use of *Phytolacca dodecandra* L. in schistosomiasis control is considered cheaper, environmentally safe, biodegradable and more readily available plant molluscicide than the currently available synthetic chemicals (Lambert *et al.* 1991; Molgaard *et al.* 2000).

The effects of *Phytolacca americana* L. on natural killer (NK) cells activity was studied by Sriwanthana *et al.* (2007). It is also reported that the plant could be clinically used for modulating immune functions of the body. Ethanolic extracts of fresh roots of *Phytolacca americana* L. was used as an emetic. *Phytolacca* mitogens, derived from the ethanolic extract of the plant were found to have a stimulating effect on murine B and T lymphocytes (Yokoyama *et al.* 1976).

Berries of *Phytolacca dodecandra* L. have molluscicidal properties due to the presence of saponins. The extracted saponins were used in schistosomiasis control programme. Members of Phytolaccaceae were used in polyherbal formulations (Nature cure bitters – NCB) due to their medicinal effects (Stanley *et. al.* 2005).

The roots and leaves of *Petiveria alliacea* L. is long used as a herbal remedy for medical conditions. It is also reported to have some anticancer properties. *Petiveria alliacea* L. is commonly called by the indigenous people of Amazon as 'anamu', apacin, apacina, apazole de zorro etc. *Petiveria alliacea* L. had a long history in herbal medicine in all the tropical countries where it grows. In Brazilian herbal medicine, it is considered as antispasmodic, diuretic, menstrual promoter, stimulant, and sweat promoter (Stolznberg and Parkhurst (1976). Herbalists and natural health practitioners use *Petiveria alliacea* L. for edema, arthritis, malaria, rheumatism and poor memory and as a topical analgesic and anti-inflammatory agent for skin afflictions. It is reported that the plants are used to reduce inflammation and pain. The plant was also reported to be used for over all immunity enhancement (Kim 2006). *Petiveria alliacea* L. was also known to reduce anxiety and fever spasms, to expel worms and to induce abortions. In large dosages it is toxic if taken internally. The root is more powerful than the leaves. In Ka'apor ethnobotany it is called 'mikur-ka'a' which means

opossum-herb and it is used for both medicine and magic (Yokoyama *et al*. 1976; Lans 2006).

The anti-inflammatory activity of *Petiveria alliacea* L. is compared to that of niflumic acid, a non-steroid compound of anti-inflammatory action. The anti-inflammatory action could be due to the presence of flavonoids, since they inhibit phospholipase A2 lipo-oxigenase. The flavonoids are known for the antioxidant properties (Ferrera and Basterrechea 1996; Murakani *et al.* 2000; Pacheco *et al.* 2006).

The antioxidant effects of the thiosulfinate derivative, S-benzyl phenyl methane thiosulfinate (BPT), of *Petiveria alliacea* L. against the cumene and methyl linoleate in chlorobenzene were studied using HPLC by Youji *et al.,* (2008). It was found that BPT provided effective inhibition with a well defined induction period under these oxidation conditions.

Allelopathic potential of *Petiveria alliacea* L. was studied by Perez-Leal *et. al.* 2005. The antibacterial activity of *Petiveria alliacea* L. was analysed using aqueous and ethanol extracts of the plant. It is also used as a remedy for whooping cough. The active principle extracted and the ointments prepared from the leaves of *Petiveria alliacea* L. showed anti-inflammatory action (Pacheco *et. al.* 2006).

In vitro analyses of *Petiveria alliacea* L. were done for the production of somatic embryos enriched with dibenzyl trisulfide (DTS). Leaf explants

were used for developing somatic embryos (Webster, *et. al.* 2008). Pharmacological effects of water extracts of the leaves of *Petiveria alliacea* L. were studied in rats. Even though the plants are known as poisonous plants no signs of toxicity were found in rats (Lopes *et al.* 2002).

Rivina humilis L. is popularly known as 'dog blood' or 'blood berry'. It is also called 'pigeon berry'. The decoction of *Rivina humilis* L. is a cure for colds and diarrhoea. Pounded leaves were used for wound dressing and to treat catarrh with *Eryngium*. It is a known remedy for eye diseases. Decoction of the entire plant cures jaundice and chest pain and berries alleviate dysentery and amenorrhoea. Fruit is reported to be the source of a dye. The root and berries are said to be poisonous (Sivarajan and Indu 1987).

1.1.5 PHYTOCHEMICAL AND PHARMACOGNOSTIC IMPORTANCE

Phytochemical characters have been classified into primary constituents (macromolecules), secondary constituents (not in direct metabolism) and miscellaneous substances (based on their natural relationship) (Hawkes 1968). Preliminary phytochemical screening of many plants indicated the presence of steroids, alkaloids, glycosides, tannins and carbohydrates in chloroform ethyl acetate and ethanolic extracts.

The phytochemical and pharmacognostic characters of Phytolaccaceae were analysed by many workers. The *in vitro* bioactivity and cytotoxicity analyses were conducted in many members of the family by Neish (1964),

Kingsbury and Hillman (1965), Lemma (1965), Owners *et al.* (1973), Spielman and Lemma (1973), Parkhurst *et al.* (1974), Yokoyama *et al.* (1976), Stolzenberg and Parkhurst (1976), Lee *et al.* (1985), Del (1988), Slaccanin *et al.* (1988), Baker and Brooks (1989), Ndamba *et al.* (1989), Storie *et al.* (1992), Appel (1993), Thilborg *et al.* (1993), Ndamba *et al.* (1994,), Thilborg *et al.* (1994), Ndamba *et al.* (1996), Lee *et al.* (1997), Peixoto *et al.* (1997), Raskin *et al.* (1997), Guiling (1998), Han *et al.* (1998), Shao *et al.* (1999), Kim *et al.* (2000), Molgaard *et al.* (2000), Yingfang *et al.* (2000), Kennedy *et al.* (2002), Lopes *et al.* (2002), Bais *et al.* (2003), Inderjit (2003), Fujii *et al.* (2004), Kim *et al.* (2005), Perez-Leal *et al.* (2005), Kim (2006). Pacheco *et al.* (2006), Girma and Johan (2007), Youji *et al.* (2008) and Webster *et al.* (2008). The mineral composition and the mineral accumulation capacity of members of Phytolaccaceae were discussed by Chi and Kim (1985), Marschner (1995), Xue *et al.* (2004), Xue *et al.* (2005), Xu *et al.* (2006), Sigrid and Willi (2008), Xianghu *et al.* (2009) and Ye *et al.* (2009).

1.1.6 VARIATION AMONG ACCESSIONS

The phenotypic variation and genetic diversity of different accessions and ecotypes of species of Phytolaccaceae were studied by Speckman *et al.* (1965), Tan and Dunn (1973), Davis (1985), Santen and Casler (1986), Dawson *et al.* (1993), Paterson *et al.* (1993), Rohlf (1993), Tinker *et al.* (1993), Thormann *et al.* (1994), Powell *et al.* (1996), Decraene (1997),

Mishra (1997), Rohlf (1998), Zhu *et al.* (1998), Semagn *et al.* (2000), Semagn *et al.* (2001) and Semagn *et al.* (2003). The morphological variability of *Phytolacca dodecandra* L. sampled from 17 localities was investigated by Semagn *et al.* (2004).

The ploidy level of *Phytolacca dodecandra* L. is unclear. It is indicated that the species is tetraploid with 2n = 4X = 36. A detailed investigation was found to be difficult due to the small size of chromosomes (<1µm). Stomatal size (guard cell length), stomatal frequency and stomatal plastid number have been used as morphological markers for identifying ploidy levels in several plant species (Speckman, *et al.* 1965; Mishra 1997).

RAPD TECHNIQUE TO ANALYSE GENETIC DIVERSITY

Unlike morphological markers, molecular markers are not prone to environmental influence and are extremely potent to portray the genetic relationship between plant groups (Brown and Langley 1979; Tinker *et al.* 1993; Schnell and Knight 1993). These markers could be used to select priority areas for conservation and provide vital information for the development of genetic sampling, conservation and improvement strategies (Wilde *et al.* 1992; Koller *et al.* 1993).

Emergence of the technique RAPD (Randomly Amplified Polymorphic DNA) for rapid, efficient and routine analysis of DNA offers a new suite of characters that promise the possibility of examining accumulated

genetic differences without the interference of environmental and seasonal variations. RAPD is generated by the amplification of the genomic DNA with a single primer of arbitrary nucleotide sequence (Welsh and Mc Clellard 1990; Williams *et al.* 1990).

Some of the studies done using RAPD in different plants include the genetic analysis of *Allium,* discrimination and verification of genotypes in *Eucalyptus*, comparative genetic diversity studies of *Theobroma cacao* L. (Wilde *et al.* 1992), genetic diversity studies in Indian *Musa* germplasm (Bhat and Jarret 1995), studies on Intra and Inter specific variations among Cruciferous species revealed by RAPD markers (Thomann *et al.* 1994), identification of cultivars and validation of genetic relationships in *Mangifera indica* L. using RAPD markers (Schnell and Knight 1993) and phylogenetic relationships and pedigree analysis in Maize using RAPD markers (Welsh *et al*. 1991). Genetic validity of RAPD markers at the intra and inter specific level in wild *Brassica* species were also studied (Phipper *et al.* 1997; Jain *et al.* 1994).

The extend of genetic differentiation among different populations of P*hytolacca dodecandra* L. sampled along altitudinal gradients was investigated using randomly amplified polymorphic DNA (RAPD) by Semagn *et al.* (2000 and 2001). 12 RAPD primers were used for principal components discriminant, correlation and stepwise multiple regression analysis. The significant correlation obtained between population means

from some RAPDS with the ecogeographical variables in the multiple regression analysis suggested that part of the RAPD polymorphism could be adaptive and responsive to environmental selection (Semagn *et al.* 2000, 2003).

The present study is concentrated on three members of the family Phytolaccaceae namely *Phytolacca octandra* L.*, Petiveria alliacea* L. *and Rivina humilis* L.*,* which are natives of the peninsular part of India. The study is aimed to evaluate the biochemical characters and Pharmacognostic peculiarities of the selected members of Phytolaccaceae giving a special emphasis on their antimicrobial activities. It is also aimed to evaluate the phenotypic and genetic variations among accessions.

1.2 OBJECTIVES

- To assay the macromolecules of the selected members;
- To study the amino acids using HPLC technique;
- To study the protein profile by SDS-PAGE method;
- To analyse the phytoconstituents of the members;
- To analyse phyto compounds by HPTLC;
- To study the antimicrobial activity of the selected members;
- To analyse phenotypic variations among accessions; and
- To evaluate genetic diversity among accessions by RAPD.

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