5. DISCUSSION

5.1 General

The major criteria selected for the study involves cellular structures and their phytochemical constituents. Cells and their structures can best be followed by understanding the biochemical processes involved in differentiation and how these are controlled by genetic, hormonal and environmental factors. Only by the integration of such information, will be able to achieve a full understanding of how cellular structure develops (Barnett, 1981). More than a decade after the above statement of Barnett (Barnett, 1981), Haigler, (1994) summed up: “it should be evident that a full understanding of cellular differentiation and the biochemical characters will be needed to know more about the cell biology, molecular biology, cell structure, biochemical contents and biophysics”. Such is the formidable challenge for a student of pharmacognosy. Dickison, (2000) pointed out that during the processing of drug producing plant parts, raw materials are routinely subjected to microscopic observations. This is especially important when dealing with powdered, section and chipped plant tissues. In addition to macroscopic examination and use of various chemical tests, microscopic evaluation is essential for the correct identity of dried pieces of plant material (raw drugs) and the detection and quantitative analysis of adulterant. He also pointed out that the
contamination of drugs by the addition of foreign organic matter not only results in a product of inferior quality but also can lead to an increased rate of spoilage and deterioration. Illegal adulteration also can occur by the complete substitution of one plant tissue for another. To maintain official standard of drug purity, the analyst must be an experienced microscopist and posses a good knowledge of the anatomy of drug plants, and it is now used in pharmaceutical institutions throughout the world. According to Dickison, (2000) when a plant part is powdered, the cells and tissues become variously dissociated and broken in to fragments, as a result, the preparation must be characterized using the evidences provided by individual cells, that is tracheary and fibrous elements, sclereids, leaf epidermal cells, trichomes, crystal or silica bodies and starch grains. By law, each commercially important drug of vegetable origin has an official definition that includes a description consisting of details of the internal structure of the plant part constituting the source of the drug. Plant based crude drugs whose botanical identity is not known are identified based on their morphological and anatomical characters. Park et al., (1995) studied the market samples of 'Man Byung Cho', based on morphological and anatomical characters of leaf midrib and leaf lamina and concluded that they belong to the leaves of Rhododendron brachycarpum and R. brachycarpum var. roseum. Yamaji et al., (1993) after studying the anatomical characters of flower stalk and xylem vessels of rhizome established
that the drug 'Spang-RtziDo-Do' is evolved from *Pterocarpus hookeri*. Mehrotra & Sharma, (1984) analyzed the various market samples of the Ayurvedic drug 'Sappan' and compared it with its genuine drug *Caesalpinia sappan*. Using morphological and anatomical parameters, they establish the genuineness of the drug. Of all the other areas, pharmacognostic and phytochemical content of certain medicinal plants and other sources fascinated the present author for the manifold problems associated with its development, identity, therapeutic content and above all the sharp diagnostic features in terms of cellular nature and chemical content.

Ayurveda and other traditional systems of medicine are getting increasing acceptance globally and are being groomed as sources of alternative safe medicine. Consequently the demand of ayurvedic drugs has been growing by leaps and bounds and ayurvedic pharmacies are mushrooming throughout the length and breadth of the country in the past few years. Indeed, with the increasing popularity and removal of international trade barriers, Ayurveda is poised for phenomenal growth in the coming years. But this also has brought in new challenges and questions that are being raised by the target group all around the world and the major issue is the quality control and standardization parameter of raw materials for the preparation of traditional medicines. The present author took it as a challenge to develop quality control parameters of certain medicinal plants against the questions by the target group.
The two potent threats being faced by the Ayurvedic industry are

1. The state of medicinal plants resource base which is used for the preparation of ayurvedic medicine

2. Standardisation of raw/crude drugs used in the manufacture of medicines

About 500 medicinal plants are widely used in the manufacture of various ayurvedic formulations. Besides their increasing demand in Ayurveda many of the plants are in great demand all over the world for plant derived chemicals, health care products, nutraceuticals, cosmetics, natural colouring and flavouring agents etc. As a result there has been a quantum jump in the volume of plant materials extracted and traded within the country and exported. More than 90% of the medicinal plants are collected from the wild. The extensive destruction of forest, conservation of land to crop base agriculture; over exploitation and unscientific extraction of medicinal plants have led to the extinction of very valuable plants and the shrinkage of habitat of medicinal plants. The non availability of genuine herbs in required quantity forces the collectors and suppliers to adulterate or substitute it with easily available and spurious drugs. This will affect the efficacy of medicines. This leads to the demand of target groups for the standardization and quality control aspect of Ayurvedic medicine.

The major criteria that can be used for the standardization of raw drugs are Pharmacognosy and Phytochemistry. These are species
specific with characters and can be exploited by developing anatomical and chemical fingerprints of each raw drugs. In the present study the author attempted to develop standards based on anatomical, histochemical and phytochemical characterization of three different raw drugs namely Citraka, Jivanti and Rasna, the reputed drugs in Ayurvedic medicines, coming under controversial group of plants already reported (Sivarajan & Balachandran, 1994).

5.2 Citraka

Citraka is a reputed drug in Ayurveda used for leucoderma and other skin diseases etc (Warrier et al., 2002). The botanical sources of Citraka according to Dymock, (1891) is P. zeylanica. According to Iyer & Kolammal (1960), in Kerala white flowered P. zeylanica which grows wild as well as the red flowered P. indica (Syn. P. rosea) which is cultivated are used as citraka. Kerala physicians have given preference to P. indica, and P. zeylanica being used only when the former is not available. According to Ayurveda there are three varieties of Citraka namely those having white, red and black flowers (Vaidya, 1982). According to Iyer & Kolammal (1960) only two varieties are in practice. In the classical text Astangahrdaya (2000) there are three varieties of this plant namely red, white and blue. Some say black instead of blue and some commentators equates with a fourth variety yellow also. The identity of the yellow variety is not known. Singh & Chunekar, (1972) in his Glossary of vegetable drugs in Brhatrasyi opines that this may be some hybrid variety and
in practice, the red and white flowered varieties are only procured in Indian medicine.

From the market study it is revealed that in Kerala the red flowered *P. indica* is in use as *citraka* but in North India and also in Ayurvedic Pharmacopoeia of India it is equated with white flowered *P. zeylanica*. The study reveals that the so called blue flowered *Plumbago* that is *P. auriculata* Lam., (Syn. *P. capensis* Thumb.) is an native of South Africa and is not used here as a medicinal plant at all. From the study it is evident that in Kerala *P. indica* is used after a detoxification process whereas in North India *P. zeylanica* is used directly without purification. *P. zeylanica* locally called *tumba codivel* (Rheede, 1690) or *vellakotuveli* is the source of *citraka* according to many authors (Kurup et al., 1979; Kapoor & Mitra, 1979; Dey, 1980; Sharma, 1983). However the red flowered *P. indica* L. is the accepted source of the drug in Kerala. This is known by the vernacular name *cettikotuveli* (Rheede, 1690) and is considered to be therapeutically more active. According to Sivarajan & Balachandran, (1994) the correct source of drug not given anywhere. In the present study the author conducted a thorough market survey throughout India especially in Kerala markets and produced a quality standards and a comprehensive profile of this plants which includes classical literature survey, taxonomical profiling, micromorphological and histological studies, histochemical studies, maceration and raw drug powder studies, phytochemical studies containing TLC, HPLC,
HPTLC & comparative statistical analysis of the two plants and also a review of pharmacological and therapeutical aspects.

According to Bhavaprakasa nigantu (1982), Dhanvantari nigantu (2005), Rajanigantu (2006) and Ashtangahryadayam (2000) the described synonymous agni, dāhuna, dāruna etc. for this, Citraka indicates the caustic action of the drug. Other name like vyālah indicates the strong action of the drug. Dārana and dāruna indicate that the drug is capable of furrowing action on the skin and the name Citrakah means multicoloured, it may be due to the burning action given to the skin.

P. indica is a shrubby perennial growing throughout India whereas P. zeylanica is a large perennial under shrub (Iyer & Kolammal, 1960; Sivarajan & Balachandran, 1994). The morphology is described in detailed by Iyer & Kolammal, (1960); Vaidya, (1982). The present author found that roots of P. indica consists of many long and fairly stout roots with few lateral branches which are of light yellow brown in colour, skin thin and surface smooth but with short transverse shallow furrows at the region of the bend. Taste acrid and odour disagreeable. Whereas in the case of P. zeylanica roots consists of nearly uniform spreading stout, cylindrical, nearly smooth and light brown externally finely longitudinally striated with numerous shallow fissures at places and scars of rootlets and with same type of taste and odour with that of P. indica. According to the present observation the cut portion of the roots of P. indica itself shows remarkable
differences in these two species ie, cortex form the bulk part which is whitish in colour and the central wood portion is narrow and darker. Whereas in *P. zeylanica* cortex is narrow and the central wood portion is broad when compared to *P. indica*. This is a macro morphological characteristic feature of these two roots.

In the histological details of roots of *Citraka* the present author reported that bast fibres are not conspicuous in polarized microscopic studies in *P. indica* and it is a well developed group in *P. zeylanica*. According to Iyer & Kolamml, 1960 well developed phloem fibres were reported in *P. indica* & *P. zeylanica* (Anonymous, 2008). In the present observation found that phloem fibre in *P. indica* is a rare feature which is contradictory to the reports of Iyer & Kolamml, (1960), Anonymous, (2008) and it may be due to the hormonal and environmental factors in which the plant grows.

The wide wedge shaped ray cells recall the nature of anomalous secondary thickening in roots and forms the wedge shaped wood region in *P. indica*. The role of medullary ray is very significant in the radial transportation of molecules. The present author observed that the broad ray is in greater number between xylem vessels and parenchyma. The constituent cell of the ray system have approximation and possible interconnection with the xylem parenchyma, xylem vessels, phloem and cortical parenchyma as revealed by the present observation. According to Lev-Vadun & Aloni, (1992) the mechanism of initiation of ray by ethylene is as follows:
rays drain ethylene from the xylem to the periphery, difference in ethylene production in xylem during the growth season can result in changes in the initiation of new rays and the occurrence of fibre associated with the rays. Occurrence of radial fibre in rays suggests a radial flow of developmental stimuli for fibre formation (Lev-Vadun, 1994). Eventhough the present author has no evidence on the actual synthesis or translocation of ethylene, the morphological evidences such as the wide rays and xylem parenchyma in *P. indica* and the associated biochemical changes and high plumbagin content has, very much variation with *P. zeylanica* supports the physiological model suggested by Lev-Vadun & Aloni (1992, 1994). Further they suggested that ethelene influences ray structure and initiation by its known negative effect on the polar auxin transport. The morphohistogenic and physiologic significance of such a net-work of symplast is enhanced by the reported study of Lev-Vadun & Aloni, (1990). A radial transportation system is maintaining the wide pitted ray cells. Hence it is hypothesized that the photosynthates from phloem to various cells is carried out through xylem ray cell network and often the cell content enter in to the tylosis of the vessel through medullary rays to xylem parenchyma and then to xylem vessel. Though it is a storage root it lack starch but more yellow plumbagin content in *P. indica*. Whereas in *P. zeylanica* starch grains are stored in ray cells and parenchyma and comparatively less plumbagin.
The present author's observation support to Iyer & Kolammal, (1960); Anonymous, (2001) & Anonymous, (2008) with regard to the presence of phloem fibres, sclereids and a lot of starch grains in *P. zeylanica*. The narrow medullary ray full of starch grain is another significant difference observed by the present author in *P. zeylanica*. In *P. zeylanica* the yellow content is comparatively less when compared to *P. indica*. Yet another observation made by the present author is that the morphohistogenic and physiological significance may be indicative of another co-factor for the production of starch in *P. zeylanica* in the place of plumbagin in *P. indica*.

The present author, using histochemical techniques confirmed that in *P. zeylanica* the inner cortical cells, medullary rays and xylem parenchyma are fully loaded with starch grains whereas it is meager in *P. indica*. However the deposition of plumbagin is more in *P. indica* than *P. zeylanica*. We are yet to have information on the biochemical factor the external factor and above all the intrinsic genetic mechanism determining the content of plumbagin, starch etc.

The present observation on maceration studies, in the case of vessels, length is higher in *P. zeylanica* when compared to *P. indica* but the width and wall thickness are more in *P. indica* compared to *P. zeylanica*. Fibre length is more in *P. zeylanica* whereas width and wall thickness of fibre is more in *P. indica*. Tailed and tailless type of vessels and fibres with pointed ends are present in both the plants.
Simple and bordered type of pits is present in *P. indica* but in *P. zeylanica* simple types of pits are present.

Powder microscopy is another parameter used to identify and distinguish the drug from its substitutes and adulterants. For example, Patel & Satakopan, (1979) distinguish *Saraca asoka* bark from its adulterants by the analysis of the powder and put forth a key for the identification of the 'Asoka' bark powder. Srivastava & Srivastava, (1988) identified the adulterants of *Catharanthus roseus*, by the analysis of powdered drug. The present author observed that the powder characters are significant, in *P. indica* and *P. zeylanica*. It shows differences in cork cells in surface & sectional view, transversely cut fragments of thick walled hypodermal cells along with cortical cells with plumbagin content and xylem vessels and parenchyma, fragments of xylem vessels with pitted thickening.

The present author's observation does not support Dutta & Mukerji (1950), Iyengar & Pendse (1962), Sharma *et al.*, (2000) findings with regard to the statement about *P. zeylanica*: medullary rays are straight, 1-6 seriate; cells radially elongated containing starch grains. The present observation points out that ray cells are uni or biseriate containing starch grains and the accompanying wood parenchyma cell also contain starch grain. (It also resembles the medullary ray) but from the present findings it is well evident that it is xylem parenchyma and broad multiseriate medullary rays that are characteristic features of *P. indica*. Phloem fibres are striated, pitted,
thick walled type and most of them are in groups of 10-15 numbers and this is also a characteristic feature of *P. zeylanica* compared to *P. indica*. The comparative histological studies carried out in these plants are meager and the author covered the available literature and discussed the major points. The present author has given special emphasis to locate lignified cells and calcium oxalate crystals present in the roots using fluorescent and polarization microscopic studies and it is the first report in this line.

Roots of *P. rosea* L. are the richest source of plumbagin, which is chemically identified as a naphthoquinone, the compound claimed to sublime at 90°C. Plumbagin is hydrophobic and insoluble in water. Plumbagin is the active principle of *Plumbago* and it has been isolated namely plumbagin, 3-chloroplumbagin, droserone (Sidhu & Sankaram, 1971), 3,3'-biplumbagin (chitranone) (Sankaram *et al.*, 1976), zeylanone and iso- zeylanone and a coumarin, elliptinone (Sankaram *et al.*, 1977). Two flavonoids and two carboxylic acids have been isolated from the ethyl acetate extract of the roots of *P. indica*. The structures of these compounds have been established as myricetin – 3, 3’, 5’, 7 – tetra methyl ether 1, ampelopsin – 3’, 4’, 5’, 7 – tetramethyl ether 2, plumbagic acid and roseanoic acid 4 on the basis of UV, IR, $^{1}$H and $^{13}$C NMR and mass spectrum studies. The two carboxylic acids 3 and 4 are reported here for the 1st time (Ariyanathan *et al.*, 2010). The roots of *P. indica* accumulate the naphthoquinone plumbagin (Thomson, 1957).
Sankaram et al., (1976) isolated six compounds from the roots of *P. zeylanica*. Plumbagin, 3- chloroplumbagin, 3, 3’- biplumbagin, elliptione and droserone are the five components. They reported the sixth compound to be a new biplumbagin, chitranone and considered it as 3,6'- biplumbagin. Zylanone and iso2ylanone, the two quinines were isolated from roots of *P. zeylanica* (Sankaram et al., 1977). HPLC analysis has now become a powerful tool for the quality control of raw plant materials. The HPLC estimations of plumbagin in *P. indica* & *P. zeylanica* were reported (Unnikrishnan et al., 2008). Lin et al., (2003) reported two plumbagic acid glucosides, 3'-o-β-glucopyranosyl plumbagic acid and 3'-o-β- glucopyranosyl plumbagic acid methyl ester along with five naphthoquinones and five coumarins isolated from the roots of *P. zeylanica*.

Kamal et al., (1983) reported the isolation of plumbagin, droserone, isoshinaolone and a new naphthalenone, 1, 2(3)- tetrahydro – 3, 3’ plumbagin from the roots of *P. zeylanica*. The nature of the new naphthalenone was elucidated by means of spectroscopic data and chemical interconversions. Gupta et al., (1999) performed extensive column and thin-layer chromatography of the ethanolic extract of the roots of *P. zeylanica* and isolated two naphthoquinones namely 3,8-dihydroxy -6- methoxy -2-isopropyl-1,4- naphthoquinone and 5,7-dihydroxy- 8- methoxy-2- methyl-1, 4- naphthoquinone.

Phytochemical study of the roots of *P. zeylanica* carried out by Gupta et al., (2000) isolated anthraquinone glycoside along with
naphthoquinone from the benzene-ethyl acetate fraction of the roots. From the spectroscopic and chemical study they identified the naphthoquinones as droserone and zylanone.

Lin et al., (2003) isolated cytotoxic naphthoquinones, plumbagic acid glucosides and coumarins from the roots of *P. zeylanica*. The two plumbagic acid glucosides are 3-O-β-glucopyranosyl plumbagic acid and 3-O-β-glucopyranosyl plumbagic acid methylester. Nguyen et al., (2004) performed the bioassay-guided fractionation of the dichloromethane extract of aerial parts of *P. zeylanica*. Hsieh et al., (2005) suggested the LC-MS/MS method for the determination of plumbagin from *Plumbago zeylanica* root extract and found that the liquid chromatographic separation of plumbagin with tandem mass spectrometric detection demonstrates an accurate and reproducible quantitation of this compound.

In *P. zeylanica* a number of other benzoquinones, including dimmers of plumbagin have been reported (Sankaram et al., 1979). Mallavadhani et al., (2002) reported the characterization of plumbagin. In *P. indica* (Dinda et al., 1992, 1999) reported 6-hydroxy plumbagin, sitosterol, stigmasterol, campesterol, plumbagic acid lactone, two flavonol methyl ethers- azaleatin, cyanin and two aliphatics- palmitic acid and myricyl palmitate.

Akella et al., (1976) reported chitranone a new binaphthaquinone from *P. zeylanica*. Gopinath et al., (2009) reported a simple, rapid, precise RP-HPLC method developed for simultaneous estimation of
plumbagin and embelin containing in different extracts. Phytochemical screening of extracts of *Plumbago zeylanica* revealed the presence of several constituents, including plumbagin, linoleic acid, palmitic acid, nonylnonanoate, stigmasterolacetate, lupeol acetate, friedelinol, lupeol, luponone, sitosterone and stigmasterol (Akella *et al.*, 1976; Dinda *et al.*, 1989; Chowdhury *et al.*, 1981; Kodithala *et al.*, 2002).

Gupta *et al.*, (1995) isolated three new compounds, nonyl nonanoate, nonyl 8-methyl-dec-7-enoate and benzyl 2,5-dihydroxy-6-methoxybenzoate, from the roots of *Plumbago zeylanica*. Plumbagin, droserone, isoshinanolone and a new naphthalenone (1, 2(3)-tetrahydro-3'-biplumbagin) were isolated from the phenolic fraction of the light petrol extract of the roots of *Plumbago zeylanica* by Gunaherath *et al.*, (1983). The main constituent of the neutral fraction was sitosterol.

All the phytochemical constituents reported by the earlier workers show a clear idea about the active principle in the two plants and that the major therapeutically active compound is plumbagin. In the present study the author attempted to study the comparative characters like moisture content, water soluble extractive, alcohol soluble extractive, ash value and acid insoluble ash, total phenolic, flavonoid and tannin contents and the comparative TLC, HPTLC and HPLC profile of these two plants.
The present authors' observation in physicochemical parameters except ash value all other parameters were more in *P. indica* compared to *P. zeylanica*. In both the case the ash value shows same result. Total phenolics, flavonoid and tannin contents are more in *P. indica* compared to *P. zeylanica*. Gupta *et al.*, (2008) reported that root of *P. rosea* is considered to be more potent than *P. zeylanica*. The present author's observation shows that chemical constituent wise *P. indica* is superior than *P. zeylanica*. Though content wise increase was observed in *P. indica*, reports on chemical characterisation and isolation of compounds are comparatively less when compared to *P. zeylanica*.

The TLC profiling and *Rf* value of the prominent bands of roots of *P. indica* and *P. zeylanica* were compared using the solvent system Hexane: Ethyl acetate. Similar type of banding pattern was observed in *P. indica* and *P. zeylanica* under 254 nm. Whereas in 366 nm at *Rf* 0.41 one particular band was missing in *P. zeylanica*. The TLC profile after derivatization also shows similar banding pattern except for some thickness of bands. Gupta *et al.*, (2008) reported the *Rf* value and TLC profiling of *P. zeylanica* using the solvent system Toluene and formic acid but no comparative reports on *P. indica*. Present author compared the TLC by using plumbagin as marker compound (a band at *Rf* 0.52) because it is present in both the plants and quantification of plumbagin were carried out using HPLC and HPTLC methods. Quantification of plumbagin was carried out using the area
under the curve method and the resultant concentration of plumbagin shows differences in *P.indica* and *P.zeylanica*. From the result it is confirmed that both the species have plumbagin compound. The percentage of plumbagin present in *P.indica* is 0.2 % were as in *P. zeylanica* it is 0.16 %. From the HPLC comparison of sample it is confirmed that *P. indica* contains more plumbagin which is being used in Kerala after purification process. Whereas in *P. zeylanica* which is being used in North India without purification have low concentration of plumbagin.

The present investigation justifies the method of North Indian ie, selection of *citraka* as *P. zeylanica* without purification, and the traditional knowledge which is being continued in Kerala ie., purification of *citraka* (*P. indica*). The present author conducted the purification process using lime water and water on hourly and day basis as per the procedure and observed that after purification process, the percentage of plumbagin reduced and found to be almost equal to *P. zeylanica*. The plumbagin at low doses gives stimulative action on nerves but at high doses it causes irritation to skin and is highly toxic and is reported to be neurotoxic, leading to paralysis and may cause death. (Anonymous, 2001; Mallahdhavi et al., 2002; Unnikrishanan et al., 2008).

The HPTLC quantification of plumbagin before and after purification process revealed that one day purification in lime water and two day purification in water gives equivalent quantity of plumbagin when
compared to *P. zeylanica* the north Indian sample. The normal traditional purification process now in practice is washing in lime water and cow dung water for one week or until the water turned clear and we found that when the days of purification increases the amount of plumbagin reduces considerably and the compound reaches 0.004% in lime water and 0.007 % in water purification. Our purification process ie., one day is enough to attain the same amount of plumbagin content found in *P. zeylanica* Mallahdhavi etal., (2002) and Unnikrishanan etal., (2008 ) reported that high doses of plumbagin causes irritation to skin and is highly toxic which leads to paralysis and ultimately death. From the present observation using HPTLC quantification, the prolonged purification process up to one week may cause high percentage of reduction of plumbagin and other chemical constituents when compared to non purified *P. zeylanica* and *P. indica* and this may affect the therapeutic efficacy of *P.indica* which is being used as *citraka* in Kerala. The present work scientifically validates the purification process of *citraka* that is mentioned in the ancient text and has produced a simple, economical and refined protocol for the purification process to get maximum therapeutic value from that particular drug.

A thorough market survey was conducted throughout Kerala to collect *citraka* and compared the anatomical and chemical profiling of the raw drug to find out the genuine sample. Using the quality standardisation parameters that we have developed in the present
work it is confirmed that all the materials are *P.indica*. Though there is no variation in the base anatomical structure, difference was observed in the deposition of starch, lignin and yellow brown plumbagin content. Agro climatic conditions and hormonal impact are directly proportional to the chemical constituents. The secondary metabolite production in plants may be subjected to considerable variation in the living plants, depending on environmental and ontogenic factors and more stable chemical characters are occurred by those closely associated with DNA composition of the species (Evans, 2002).

In view of its medicinal and toxic properties, the plumbagin is reported to have anti-cancer property. The biological studies like bio-activity, cytotoxicity, anti-fertility, anti-fungal, anti-inflammatory, antibacterial, antioxidant, anti-insecticidal and CNS activity have been reported on *P.zeylanica* by Goutam *et al.*, (2007). Whereas the pharmacological studies on *P. indica* is less. The major biological activities are anti-fungal (Valsaraj *et al.*, 1997) anti parasitic (Paiva *et al.*, 2003), anti-fertility activity and antitumor activity (Sheeja *et al.*, 2009).

The biological activity of plumbagin, the most active naphthoquinone derived from the species of *Plumbago, Drocera* and *Diospyrus* had been widely studied. In small doses it is a sudorific and stimulates the central nervous system whereas in large doses may cause death from respiratory failure and paralysis (Sermwut Kaewbumrung,
The pharmacological activity of plumbagin compound are antitumor activity (Kuo et al., 2006, Hsu et al., 2006) anti-inflammatory (Checker et al., 2009), antimalarial (Paiva et al., 2003) anti bacterial (Park et al., 2006), Mutagenic activity (Kato et al., 1994) anti-infertility activity (Kini et al., 1997), abortiflcient activity (Premakumari et al., 1977), reproductive toxicity (Chowdhury et al., 1982), Cardiotoxic (Itoikawa et al., 1991), hypolipidemic and anti antherosclerotic effect (Sharma et al., 1991) and microsomal enzyme activity (Muto et al., 1987).

From the literature survey it is found that plumbagin compound is a biologically active, potent compound used for several activities. Hence the author has come to a conclusion that whether P. indica or P. zeylanica the plumbagin content is one of the important therapeutic compounds. A lot of toxicological work can be conducted in both the species. The root of P. zeylanica has numerous therapeutic uses. The root is known to be abortifacient at dose dependent rates and to have vesicant properties. It is used as appetizer and in the treatment of dysentery, diarrhea, diuretic, expectorant, piles and peptic ulcers. The root paste applied topically for filarial leg is found useful. It is used topically for early as maturation, rupture and healing of abscess. The root powder taken orally along with honey gradually reduces hypercholestraemia and improves blood formation. It is used to reduce obesity, vitiligo, splenomegaly, hepatomegaly and ascitis. It is also used to relieve
coryza (running nose), hoarseness of voice and sore throat. It is used in the form of local applications for leucoderma, scabies, psoriasis, symptoms of leprosy and allied skin diseases. The decoction of the root is useful in checking and preventing spermatorrhoea. The present study also provides an opportunity to investigate and establish the status of *P. zeylanica* to find its utilization in different ailments. High dose of plumbagin results the abortifacient and hemorrhages caused by the inhibition of vitamin K activity (Premakumari *et al.*, 1977; Chowdhury *et al.*, 1982). In *P. zeylanica* the plumbagin content is in lower amount than *P. indica*. Low dose is recommended in most of the pharmacological data so far obtained. Finally it can be concluded that these studies also initiate the researchers who are in this field for further pharmaceutical studies and therapeutic uses on *citraka* for total drug evaluation (Chetty *et al.*, 2006). Traditional uses of *P. zeylanica* was reported by Singh *et al.*, (2004). Hence *P. zeylanica* can be used as a source plant of *citraka* which is available in Kerala and can be used without purification because it contains less amount of plumbagin.

The data obtained from this study would be useful in identification of roots of *P. zeylanica* and can serve as quality standard. The histological and histochemical parameters and quantification of the marker compound plumbagin done by TLC, HPLC and HPTLC are found to be a good quality checking parameter and the plumbagin content in the order of *P. indica* (0.2%) ≥ *P. zeylanica* (0.16%), which
can also be considered as an additional parameter for quality checking of these roots. The data evolved can be considered for laying down the pharmacopoeial standard for the roots of these plumbago species.

5.3 Jivanti

Jivanti, the drug is considered to have the property to bestow health and liveliness to the consumer. Caraka treats it as an important rasayana drug, capable of maintaining the youthful vigour and strength. The commentators Dalhana, (1982) has clearly identified 'Dodi' for jivanti. Jivinti is the best among the vegetable by caraka. In Gujarat people use this jivanti as pot herb and is consider very valuable in T.B and eye diseases. According to Vaidya, (1982) jivanti is equated with L. reticulata, a twining shrub, but in Bengal jivanti is equated with Dendrobium macræi. It is a golden coloured orchid an epiphytic herb so it can be distinguished easily. The whole plant is golden yellow in colour. It is also called suvarna jivanti in Bengal. Vaidya, (1982) also reported that if yellow latex is obtaining then it is considered as suvarna jivanti, and equated Holostemma annulare which is very similar to L. reticulata the true jivanti. So he set aside the claim of Bengal Kavirajs over the Dendrobium macraei as suvarna jivanti and state that jivanti is pot herb par excellance and orchid cannot be used as vegetables. Classical literature mentioned six different varieties of this drug which probably represent different plants (Mooss, 1976; Kolammal, 1979). Mooss, (1976) however
recognize two varieties, jivanti and brihat jivanti but in actual practice this discrimination is not in recognized. Kolammal, (1979), Chunekar, (Bhavaprakasa nigantu, 1982) describes that the plant is a good vegetable (sakasreshta) having a copious exudation (madhusrava, payasvini) which has a golden yellow colour (suvarnika, svarnavarnini, svarnalata), yellow flower (hemapuspi) and (arkapuspi) flowers those resemble calotropis gigantea. Botanical identity of this drug is highly disputed. Nadkarni, (1954), Chopra, (1956), Dey, (1980), Chunekar, (Bhavaprakasanigantu, 1982) equated jivanti to an orchid. The whole plant being golden yellow in colour being used as suvarna jivanti in Bengal and some other parts of India (Vaidya, 1982). Yet others consider a asclepiadaceae plant L. reticulata as source of jivanti (Singh & Chunekar, 1972; Chunekar, (Bhavaprakasa nigantu, 1982).

Jivanti according to Parameswara is the adapathiyam of Kerala physicians, which has been identified as Holostemma ada-kodien. Rheede’s, (1689) description and illustration of the plant under the name adakodien says that roots of this plant are being used as jivanti in Kerala from time immememorial and this may be the variety of arkapuspi mentioned by Caraka since its flowers are very similar to that of Calotropis gigantea. Hence the controversy is between jivanti- L. reticulata and Holostemma ada-kodien where as Dendrobium macraei is clearly equated with suvarna jivanti. These two plants (H. ada- kodien and L. reticulata) belonging to the same
family Asclepiadaceae. The roots are used in the treatment of skin infections, wounds (Sarin, 1999; Sharma et al., 2001) burning sensation in the body, emaciation and general debility (Kurup et al., 1979). The roots are sweet, refrigerant, ophthalmic, emollient and galactogogue. Jivanti is cold, sweet, aphrodisiac, rejuvenative and easy of digestion. It promotes health and vigour, improves voice, and cures eye disease, cough, dyspnoea, fever and burning sensation. Dysentery, nightblindness, poisonous affections and tuberculosis are also relieved by the use of the drug (Kolammal, 1979; Chunekar (Bhavaprakasanghantu, 1982). The root is the official part, used in the preparations like Jivantyadi ghrtam, Manasamitravatakam, Balarishtam, Anutailam etc (Sivarajan & Balachandran, 1994).

In the present study the author conducted a thorough market survey throughout India especially in Kerala markets and produced a quality standards and a comprehensive profile of this plants which includes classical literature survey, taxonomical profiling, micromorphological and histological studies, histochemical studies, maceration and raw drug powder studies, phytochemical studies containing TLC, HPLC, HPTLC & comparative statistical analysis of the two plants and also a review of pharmacological and therapeutical aspects.

In the present study the micromorphological features reveals that in the case of H. ada-kodien roots are fairly long, at times reaching a length of one metre or more irregularly bent, nearly cylindrical
through out its major part and gradually tapering towards the tip, and are light yellowish brown in colour. The surface is fairly smooth except for the presence of a few scars of rootlets. The thickness of roots and their texture varies according to age and amount of storage material (starch) present. Generally most of the roots are one centimetre in thickness, tuberous and starchy. The transversely cut surface of a fresh root about 1.5 cm. in diameter, shows a yellowish central core of wood occupying about the diameter (7 to 9 mm.) of the root en-circled by whitish (starchy) middle region with a width of half the radius (3 to 4 mm) and at the extreme periphery a thin border of light (yellowish) brown in colour. In between the outer corky border and the whitish middle zone as a sort of separating line, can be made out of numerous brownish dots arranged to form a circle. In L. reticulata roots are cylindrical and 5 to 7 cm in length, 1 to 3 cm in thickness. Surface of the root is light brown to greyish brown with white or buff coloured longitudinal ridges and furrows. The surface is tough in nature. Fractured surface is creamish and horny, odour and taste indistinct. The transversely cut surface of a fresh root about 3 cm. in diameter shows central core of wood.

Kolammal, (1979) reported the use of Holostemma ada-kodien as the source plant of jivanti in Kerala and described the micromorphology of H. annulare. But nothing is mentioned about the histological details of L. reticulata. The micromorphological details of L. reticulata has been reported by Sharma et al., (2001), Anonymous, (2008). The
present study shows that stone cells are present only in outer
cortical layers, and the medullary rays are uni to biseriate and all the
remaining cells are found to be axial parenchymatous cells. Gupta,
(2005) reported only the stem part as jivanti instead of roots.
The present observation on histochemical details of Holostemma ada-
kodien shows starch grains which are large, simple and compound
type are present in cortex, phloem region, medullary rays and xylem
regions. In L. reticulata the starch grains are small, simple and
compound type and are present in the cortical region and medullary
rays but not in phloem region. Both these plants contain stone cells
and crystals of calcium oxalate. Intraxylary phloem is observed in
both the plants. From the present observation the major histological
differences observed in Holostemma ada-kodien are; the outer most
tissue, which is the cork or phellem is composed of four to six or
more rows of thin walled rectangular to tangentially elongated cells
that vary from 33 to 60μ in length (tangential) and 12 to 21 μ in
width (radial). The cells appear devoid of contents. The walls of the
outermost or peripheral row of cells are light brown in colour. In L.
reticulata outermost region, the cork consists of rectangular and
tangentially elongated cells. A distinct phellogen is not evident in H.
ada-kodien. Phellogen is 1 to 2 layered in L. reticulata. A narrow zone
of phelloderm is present in H. ada-kodien and is composed of oblong
or rectangular, tangentially elongated thin walled cells most of which
contain starch grains and some of the cells of the innermost rows
contain rosette crystals of calcium oxalate. Adjoining this layer are numerous, fairly prominent groups of stone cells arranged in a circle, but not forming a continuous ring. All these characters are found to be diagnostic from the present study. In the case of *L. reticulata* phelloderm consists of thin walled parenchyma cells most of which contain starch grains and some of the cells of the innermost rows contain prismatic crystals of calcium oxalate and groups of stone cells. Stone cells are arranged in a circle, but do not form a continuous ring. In *H. ada-kodien*, the phloem is made up of sieve tubes, companion cells, parenchyma and fibres, radial as well as tangential narrow strips of compressed or collapsed elements and several phloem rays (uniseriate as well as biseriate) that radially traverse the entire tissue. The ray cells of the phloem appear slightly larger than neighbouring cells of phloem, and are also heavily packed with starch grains as like parenchymatous cells outside the phloem. In *L. reticulata* the phloem is made up of sieve tubes, companion cells, parenchyma and fibres and stone cells being traversed by uni to multiseriate medullary rays, groups of fibres and stone cells are present in the outer phloem region. Stone cells are about 60μ in length and 20 μ in width.

In *H. ada-kodien* the xylem (wood) which is at the centre constitutes the major part of the root. It is not very hard or woody on account of the meagre or poor development of thickwalled lignified elements. The major part of xylem is composed of thinwalled parenchyma that
forms broad radial strands. The thick walled lignified elements occur as comparatively small areas or patches, with one group located at the centre of the root. The amount of proportion of lignified and unlignified xylem elements and their position except that of the central lignified core differs in different roots. The core or patch of wood situated in the centre of the root shows a diarch primary xylem surrounded by secondary xylem and consists of few vessels, wood fibres and lignified xylem. These occur as irregularly wedge shaped radial strips of varying width arranged towards the periphery to form a ring and also in a scattered manner amidst the large and broad zones of un lignified thin walled xylem parenchyma. In *L. reticulata* the xylem (wood) which is at the centre constitutes the major part of the root. Xylem is represented by vessels, tracheids, fibres, parenchyma, intraxylary phloem and uni to multi seriate medullary rays. All xylem elements except interxylary phloem is thickwalled and lignified. From the study both root shows anomalous secondary growth i.e., production of parenchymatous cells initiation of xylem in most of the cells. This result discrete xylem core. The nature of discretion of xylem is different types in *L. reticulata* and *H. ada-kodien*. Gupta & Kapoor (1971) and Anonymous, (2008) reported 'islets' of intraxylary phloem embedded in *L. reticulata* wood. Whereas Kolammal, (1979) reported intraxylery parenchyma in *H. annulare*. In the present study interxylery phloem and thin walled axial parenchyma are observe in *Holostemma ada-kodien*. In *L. reticulata*
prismatic crystals of calcium oxalate and starch grains are observed in medullary rays. There are no reports regarding the presence of crystals in medullary rays. The polarization, fluorescent and powder studies reveal that stone cells are highly lignified in *Holostemma ada-kodien* compared to *L. reticulata*. All these characters are not mentioned in the earlier reports on these plants by Gupta & Kapoor, (1971); Warrier et al., (2001); Sharma et al., (2001); Anonymous, (2008).

Chemical characterization studies are more in *L. reticulata* compared to *Holostemma ada-kodien*. *Leptadenia reticulata* has gained attention to some extent from the chemical point of view. In a preliminary phytochemical investigation of the species, Verma & Agarwal, (1962) recorded the percentage of ash and presence of sugars, glycosides, steroids and terpenes. Waxes and steroids of the plant excluding leaves were characterised by Murti & Seshadri, (1944). From the leaves and twigs, the hentriacontanol, amyrins, stigmasterols and two flavonoids were isolated (Krishna et al., 1975) and from the follicle, β-sitosterol, quercetin and its glycoside (Subramanian, 1968; and Subraranian and Nair, 1968). Rao & Malviya, (1966) reported the presence of sugars, amino acids and several enzymes in the latex. An easy and economical method was suggested by Anjaria, (1980) for extraction of non-saponifiable compounds. Stigmasterol and tocopherols were isolated in pure form and identified. *Leptadenia reticulata* contains a triterpenoid,
leptadenol (C30H50O). It also contained n-triacontane, acetyl alcohol, β-sitosterol, β-amyrin acetate, lupanol 3-O-diglucoside and leptidin (Noor et al., 1992). Presence of α-amyrin, lupeol, β sitosterol, alanine, aspartic acid, glycine, serine, threonine and valine was reported from the ethanolic extract of roots of *H. ada-kodien* (Ramiah et al., 1981). Comparative evaluation of the amino acids and terpenoid compounds in the root tubers and *in vitro* induced callus of *H. ada-kodien* was carried out. Results on comparative analysis of amino acids showed no alteration in the primary metabolism in the callus. The metabolism of production of terpenoid compounds in the callus, however, showed alteration (Karmarkar et al., 2001). Chemical compound reported on *H. ada-kodien* are meager compared to *L. reticulata*. In both the roots the common compounds observed are β sitosterol, lupeol and α-amyrin. In the present investigation β sitosterol is found to be common in both the roots. In the present study author selected β sitosterol for the comparative phytochemical profiling and quantification of that particular marker compound in both the species. Five physicochemical parameters were studied in both the roots. Moisture content, water soluble extractive, alcohol soluble extractive, ash value and acid insoluble ash. From these five parameters the values of moisture content, water soluble extractive & acid insoluble ash are higher in *H. ada-kodien* and the remaining, alcohol soluble extractive & ash value is higher in *L. reticulata*. These
physicochemical values of *L. reticulata* has been reported by Anonymous, (2008) and it is within the reported limit. Whereas the physico chemical parameters of *H. ada-kodien* and its comparison with *L. reticulate*, this is the first report. The present author observed that total phenolics, flavonoid and tannin contents are more in *H. ada-kodien* than in *L. reticulata*. So far no reports are available regarding the comparative quantification of total compounds present in these two roots and the study reveals that total content wise *H. ada-kodien* is superior than *L. reticulata*. The TLC profiling and \( R_f \) value of the roots of *H. ada-kodien* and *L. reticulata* were compared using the solvent system Toluene: Ethyl acetate. From the chemical comparison, TLC profiles and \( R_f \) value of prominent band using methanolic and chloroform extract reveled that only a single compound is common in both the root and using the marker compound it is identified as \( \beta \)-sitosterol and all other bands were different in these two roots. The chloroform and methanolic extract comparison of both the root at 254 nm and 366nm shows that *H. ada-kodien* contains 3-4 additional bands when compared to *L. reticulata*. But after derivatization seven compounds were found to be common in TLC profile of chloroform extract and five compounds common in TLC profile of methanolic extract. In all the cases the marker \( \beta \)-sitosterol is found to be the prominent band. The HPLC quantification of \( \beta \)-sitosterol was carried out using the area under the curve method. The concentration of \( \beta \)-sitosterol shows
significant difference in both the roots. The concentration of \( \beta \)-sitosterol in *H. ada-kodien* is 0.735% and in *L. reticulata* is 0.018%.

In both the plants the common biologically active compound was found to be \( \beta \)-sitosterol. We compared the HPTLC densitometric scanning to find out the percentage of \( \beta \)-sitosterol. In Methanolic extract of *H. ada-kodien* the percentage of \( \beta \)-sitosterol is calculated as 1 gm sample contains 0.00157 gm of \( \beta \)-sitosterol and in *L. reticulata* 1 gm sample contain 0.0002348 gm of \( \beta \)-sitosterol. Whereas in chloroform extract 1 gm sample of *H. ada-kodien* contain 0.0009684 gm of \( \beta \)-sitosterol and in *L. reticulata* 1 gm sample contain 0.000371 gm of \( \beta \)-sitosterol. So far no reports are available regarding the comparative quantification of \( \beta \)-sitosterol in these two roots and the above findings are reported for the first time.

A thorough market survey was conducted throughout Kerala to collect *jivanti* and compared the anatomical and chemical profiling of the raw drug to find out the genuine sample. Using the quality standardisation parameters that we have developed in the present work, it is confirmed that all the materials are *H. ada-kodien*. Though there is no variation in the base anatomical structure, difference was observed in the deposition of starch and lignin. Agro climatic conditions and hormonal impact are directly proportional to the chemical constituents. The secondary metabolite production in plants may be subject to considerable variation in the living plants, depending on environmental and ontogenic factors (Evans, 2002).
Anatomical studies of the market samples show differences in the depositions of lignin and starch according to the place of collection. The sample collected from Kollam shows adulteration when compared to anatomical characters of genuine *H. ada-kodien*. From the thin layer chromatographic profiles developed for the genuine drug and the market samples keeping β-sitosterol as marker compound, it is well clear that except the sample from Kollam all the other samples are the genuine *H. ada-kodien* for the drug *Jivanti*. The samples from Kollam was an adulterant which is identified as *Pergularia* a member of Asclepiadaceae. In the case of *Jivanti* materials were subjected to anatomical and chemical comparison using the standard procedures and reveals that market samples show differences according to the place of collection. The sample collected from one district is an adulterant and the anatomy is different from the genuine sample. From the Kerala market survey it is calculated that about 90% of the raw drug *Jivanti* were found to be *Holostemma ada-kodien*, the raw drug sources of *Jivanti* used in Kerala and 10% were found to be adulterant. The present study on market survey sets at rest to the speculiation of the probable variation in chemical constituents induced by the season and environment. The sample collected from all over Kerala showed hardly any variation in constitution this indicates that climate does not affect much in the chemical constituent of the plant and the advantage of this finding is that the collection of the plant
can be done in any season or environment. Mammen et al., (2010) studied the seasonal and geographical variation in chemical constituents of *L. reticulata* keeping coumaric acid as marker and found no effect on the synthesis of phytochemicals in plants and the present author agrees with the report of Mammen et al., (2010) in the case of *L. reticulata* keeping β-sitosterol as a marker compound. The major biological activity of β-sitosterol, one of the active compound present in the plant *jivanti*, is hypcholestolomic action on experimental animals and man. β-sitosterol is a cholesterol-lowering compound of modest to moderate efficacy and seems remarkably free from side effects for the patient with hypercholesterolemia (Subbiah, 1973). A study by Grundy & Mok, (1976) showed that intake of β-sitosterol as low as 3 gm per day inhibited absorption of cholesterol to a maximum degree and much larger doses do not cause a further significant decrease in absorption of cholesterol. From this finding the author considers that phytosterols in a sense can be a form of diet therapy rather than drug treatment. β-sitosterol is also used for boosting the immune system and preventing colon cancer as well as for gall stones the common cold and flu (influenza), HIV/AIDS, rheumatoid arthritis, tuberculosis, psoriasis, allergies, cervical cancer, fibromylgia, systemic lupus erythematosus (SLE), asthma, hair loss, bronchitis, migraine, head ache and chronic fatigue syndrome. Some men use beta- sitosterol for enlarged prostrate (benign prostatic hyperplasia or BPH). Some women use it for
symptoms of menopause. It is also used for enhancing sexual activity. Marathon runners sometimes use β-sitosterol to reduce pain and swelling after a run, some other apply β-sitosterol to the skin for treating wounds and burns (Subbiah, 1973).

In foods, β-sitosterol is added to some margarines that are designed for use as part of a cholesterol-lowering diet and for preventing heart diseases. The federal food and Drug Administration (FAD) allows manufactures to claim that foods containing plant sterol esters such as beta-sitosterol are for reducing the risk of coronary heart disease (CHO). This rule is based on the FDA's conclusion that plant sterol esters may reduce the risk of CHD by lowering blood cholesterol levels. Although there is plenty of evidence that beta-sitosterol does lower cholesterol levels, there is no proof that long term use actually lowers the risk of developing CHD (www.webmd.com).

The roots of *H. ada-kodien* reported to possess cooling, alterative, tonic and lactative properties. The roots are also used in diabetes, gonorrhea, coughs and stomach ache (Anonymous, 2001). Janapati *et al.*, (2009) reported the antidiabetic activity of *H. ada-kodien*. The alcoholic extract of *H. ada-kodien* significantly lowered the blood sugar of hyperglycemic rats.

Anjaria *et al.*, (1975) reported the lactogenic property in *L. reticulata*. Jaytilak *et al.*, (1976) reported that a herbal preparation with *L. reticulata* exert beneficial effects on the gametogenic and androgenic functions of the testes of animals. Anjaria & Gupta, (1967)
conducted the acute toxicity studies of *L. reticulata* and reported no apparent changes to liver, kidney and heart and also reported that it shows antibacterial activity against gram-ve and gram+ve bacteria. Janapati *et al.*, (2009) studied the antidiabetic and toxicity studies of *Holostemma* and reported a dose of ≤ 500 mg/kg was non toxic and at 200 mg/kg body weight did not significantly suppress blood glucose level and he also reported the presence of alkaloid, flavonoid, flavanone, tannins, terpenoids and carbohydrates in *Holostemma*. From the literature survey it is found that both the plants are non toxic and doesn’t show any side effects. From the present study the presence of β-sitosterol in both the plants was quantified using both HPLC & HPTLC methods and the percentage of β-sitosterol is 0.73% in *H. ada-kodien* & 0.018% in *L. reticulata* and the proportionate increase is observed in HPTLC analysis. Taking in to the consideration the therapeutic value of β-sitosterol though there is no much reports on *Holostemma*, the percentage of β-sitosterol is found to be higher that shows light towards the therapeutic efficacy of the plant *H. ada-kodien* which is being used in Kerala for the preparation of *Jivantyadi ghrtam, Manasamitravatakam, Balarishtam* and *Anutailam* etc. in the same place *L. reticulata* is used in North India. *Jivanti* is one of the reported ayurvedic drug used for its rasayana properties. The correct identity of this drug however is not ascertained. Both *L. reticulata* of North India and *Holostemma* of Kerala physicians fully satisfies the claim attributed to these drug
and found beneficial in the disease for which these are prescribed. Kolammal, (1979) owing to the insufficient description of identifying characters in the old treatise of Ayurveda the problem has become more confusing. The present investigation has clearly brought out all the diagnostic macro, micro and powder characters. TLC and HPTLC quantification of marker compounds including the quantitative values, percentage extractives, ash values, polarization and fluorescent characters by which the drug L. reticulata and H. ada-kodien may be easily distinguished from other adulterants. The former (L. reticulata) may be accepted as jivanti, for it having white latex in North India while the later one in Kerala because of its calotropis like flower (arkapuspi) and yellow coloured latex as described in classical text. The present study also provide opportunity to investigate and establish further pharmacological equivalence of these plants and the presence of common class of compound like aminoacids, triterpenoides, alkaloids, flavanoids, tannins, flavones and pregnanes also shows the common characters of both these plants. From the study it is concluded that the Holostemma which is used in practice is having high therapeutic active compound compared to L. reticulata. The common therapeutic reports are galactogogue prevention of abortion and as a health tonic. Hence H. ada-kodien can also be used as substitute for jivanti.

5.4 Rasna

Rasna is another highly controversial drug and in Sanskrit
description several synonymous were given to this drug and the
given synonymous are inadequate to sort out the botanical source
satisfactorily. Caraka included rasna in the Vayasthapana varga
that are capable of maintain the youthful vigour and strength.
Kurup et al., (1979), Kirtikar & Basu, (2006), Vaidya,
(Astangahrdayakosa,1936), and Dey, (1980) equated rasna with
Vanda tessellata. Singh & Chunekar, (1972) equated Pluchea
lanceolata as the real source of rasna. In Vaidya, (1982) supported
this view. A number of widely different plants are equated with rasna
by different people. Amarakosam, (2008) reported that nakuli and
gandha nakuli are often treated as synonymous of rasna. Singh &
Chunekar, (1972) equated the synonym sugandha, gandhanakuli,
sariba and nakuli to rasna. The various authors of ayurvedic text
Dutta, (1877), Sircar, (1942), Dwived, (1949) and Indian
Pharmacopoeia (Anonymous, 1966) frequently use the Sanskrit name
rasna for Vanda tessellate, P. lanceolata and A. galanga.
Raghunathan & Mitra, (1982) suggests that the Vaidyas of South
India consider A. galanga Willd. to be rasna as one of the synonyms
of rasna is elaparni that is the plant bearing leaves resembling those
of ela. It’s another synonym sugandha is taken by them to ascribe to
the fragrance of roots of A. galanga. According to Vaidya, (1982) the
greater part of North West India rasna is supported to be P.
lanceolata. According to Warrier et al., (2002) the Kerala publications
like Osadhininigantu, Ayurvedavisvakosam gives the Malayalam term
cittaratta and peraratta for nakuli and gandhanakuli respectively
(Amarakosam, 2008). Though there is such a lot of confusion in the
identity of plant in Kerala, the rhizomes of A. galanga is used as
rasna and according to Warrier et al., (2002) & Sivarajan &
Balachandran, (1994) both A. galanga and A. calcarata are available
in the Kerala market as rasna. Nair et al., (1982) made a survey
about the rasna in South Indian markets and identified A. galanga
locally called peraratta with aromatic odour and the other less
aromatic A. calcarata as cittaratta or aratta. The two species of
Alpinia that are being used as the drug sources of Kerala, and the
North Indian rasna P. lanceolata were taken into consideration as
rasna for the present comparative studies.

In the present study the author conducted a thorough market survey
throughout India especially in Kerala markets and produced a
quality standards and a comprehensive profile of this plants which
includes classical literature survey, taxonomical profiling,
micromorphological and histological studies, histochemical studies,
maceration and raw drug powder studies, phytochemical studies
containing TLC, HPLC, HPTLC & comparative statistical analysis of
the two plants and also a review of pharmacological and
therapeutical aspects.

The comparative morphology of plant as well as individual species is
described seperatly by Vaidya, (1982) and Sivarajan &
Balachandran, (1994). But Raghunathan & Mitra, (1982) described the morphology of *P. lanceolata* and *Vanda tessellata*. A comprehensive comparison of these three plants *A. galanga*, *A. calcarata* & *P. lanceolata* were reported in the present study. The micromorphological comparison of these 3 plants clearly indicates marked variation. In *A. galanga* the rhizome is cylindrical, branched, 2 to 8 cm in diameter, longitudinally ridged with prominent rounded warts marked with fine annulations: scaly leaves arranged circularly. Externally reddish brown, internally orange yellow in colour, fracture hard and fibrous, surface rough, odour pleasant and aromatic, taste spicy and sweet. In the case of *A. calcarata*, rhizomes are horizontal and branched, 5 to 10 mm in diameter cut end circular in outline. Externally deep brownish orange internally pale buff to light brown in colour and are covered by the dried leafy bracts. Fracture is very tough uneven and fibrous, odour pleasant aromatic, taste pungent.

In the case of *P. lanceolata* root is deeply penetrating 1 to 5 mm in diameter, somewhat twisted and gradually tapering. The external surface of the young root is white, while the mature one is light brown to dark brown in colour and the internal surface is brownish. Odour is indistinct and the fracture is short, taste slightly bitter.

Raghunathan & Mitra, (1982) studied the histological parameters of *P. lanceolata*. Anonymous, (2006) reported the preliminary anatomical studies of *A. galanga* and they equated the name to *Kulanjan* instead of *rasna*. Whereas Gupta, (2003) reported the plant
A. galanga as Malayavaca. Anonymous, (2008) describe the plant A. cacarata as Granthimula. The present author carried out a comparative anatomical study to distinguish these three rhizomes. In A. galanga cortical cell consists of thin walled, polygonal and parenchymatous. Sandy crystals present, bundle sheaths are not much lignified and it partially encircles the bundles, which are present in the inner region of the endodermal layer, vascular bundles are scattered and oleoresin cells are present. Oleoresin cells are more in number in A. galanga. Starch grains are simple, elongated and oval, comparatively more in number, but in the case of A. calcarata, thin walled, polygonal and parenchymatous cortical cells are seen, bundle sheath cells are highly lignified and sclerenchymatous which completely encircles the bundle, present on both sides of the endodermal layer. Vascular bundles are scattered and oleoresin cells are less in number in A. calcarata when compared to A. galanga. Starch grains are simple, rounded or oval, less in number. In P. lanceolata the outer most region consists of thin and thick walled cells arranged in a storied condition. Large rosette crystals are present in P. lanceolata. Schizogenous cavities are seen in the cortical region, bundle sheath absent. Most of the vascular bundles are arranged in a ring some bundles are seen solitary or in groups of 3-5. Resin containing cells are more in number. Starch grains are large spherical or ovoid type.
The present observation points out that *P. lanceolata* belonging to Asteraceae and it comes under dicots and the root structure is specific and vascular bundles are arranged in ring with a central large parenchymatous zone. Whereas the other two plants coming under Zingiberaceae, a monocot plant and the rhizome TS shows specific characters and vascular bundles are scattered in the cortex and stellar region without any definite arrangement. Among these two rhizomes the distinguishing characters are in *A. galanga*, vascular bundles are partially encircled by the bundle sheath where as in *A. calcarata* highly lignified sclerenchymatous bundle sheath completely encircle the vascular bundle which is present in both inner and outer region. The shape of the starch grains are specific to *A. galanga, A. calcarata* and *P. lanceolata*. Plenty of rosette crystals of calcium oxalate are present in *P. lanceolata* whereas only sandy crystal is observed in other two plants. Reticulate and spiral vessels are a common feature. The powder characters are specific to *P. lanceolata* than the other two plants. The major powder characters of *A. galanga* consists of simple elongated starch grains; vessels with reticulate and spiral thickening; fragments of groups of longitudinally cut fibers; fragments of surface view of cork cells; fragments of longitudinally cut and cross sectionally cut view of cork cells, oleoresin cells. Fragments of cross sectionally cut view of parenchyma cells with starch grains in scattered condition; longitudinally cut fragments of
fibers; surface view of cork cells; oil globules; fragments of xylem tracheids with reticulate thickening are seen in the powder of *A. calcarata*. In the case of *P. lanceolata*, powder shows fragments of cork cells in surface view; fragments of vessels with reticulate thickening; fragments of vessels along with parenchyma with starch; cross sectionally cut view of thick walled parenchyma with starch; simple, large starch grains; rosette crystals of calcium oxalate and fragments of reddish brown deposits are scattered throughout the powder. The polarization and fluorescent microscopic studies gives a clear idea of calcium oxalate crystal and lignified cells present in these three plants.

From the literature survey nobody has conducted a comparative chemical profiling of these 3 plants and the present observation are the first report in this line. The physicochemical parameters like moisture content, water soluble extractive, alcohol soluble extractive, ash value and acid soluble ash were studied. From these five parameters moisture content is higher in *A. galanga* than *A. calcarata* and *P. lanceolata*. Water and alcohol soluble extractive and acid insoluble ash are more in *A. calcarata* than *P. lanceolata* and *A. galanga* respectively. Whereas ash value is more in *P. lanceolata* than *A. galanga* and *A. calcarata*. Comparing the total chemical compounds like phenolics, flavonoid and tannin reveals that *P. lanceolata* having highest value than the other two species. From the present study the author found that the total phenolic content is
very high compared to other two plants and in the light of antioxidant property of phenolics and tannins it is correlated to the high free radical scavenging property of *P. lanceolata* when compared to other plants.

The chemical constituents present in these three plants are found to be different in most of the cases. *A. galanga* root contains three different compounds: campheride, galangin and alpinin, from the green rhizomes, a pale yellow volatile essential oil (one of the important constituents of the drug) with a pleasant odour can be obtained on distillation. This oil contains 48 per cent of methyl cinnamate, 20 to 30 per cent of cineole, camphor and probably d-pinene (Chopra *et al.*, 1957). Itokawa *et al.*, (1987) reported the anti-tumour compounds, the phenyl propanoids 1'-acetoxy chavicol acetate and 1'-acetoxy eugenol acetate from the rhizomes of *A. galanga*. Zheng *et al.*, (1993) isolated ethyl trans-cinnamate and ethyl-4-methoxy-trans-cinnamate from root oil of *A. galanga*. Barik *et al.*, (1987) isolated two phenolic constituents, p-hydroxy cinnamaldehyde and (di-(p-hydroxy-cis styryl) methane from the chloroform extract of rhizomes of *A. galanga*.

Chemical examination of *A. galanga* from Malaysia showed the presence of 1,8 cineole, α pinene, bornyl acetate, geranyl acetate and major monoterpenoids with large amounts of α-bergamotene (10.7%), trans- β farnesene (18.2%), curcumene (1.9%), β-bisabolene (16.2%), β-sesquiphellandrene, caryophyllene oxide
(2.5%) along with methyl eugenol, eugenol acetate, chavicol and chavicol acetate (De poorter et al., 1985). The volatile oil of rhizomes of *Alpinia galanga* was analyzed. Sixteen compounds accounting for 87% of the essential oil were identified. The major constituent was zerumbone (44.8%). This is the first report of zerumbone in *A. galanga* rhizome oil of Sri Lankan origin (Arambewela et al., 2007). The main constituent of galangal extracted by hydrodistillation was methyl chavicol. In solvent extracts it was fraesol (Baydar et al., 2004).

Phan et al., (2007) studied the chemical composition from *A. henryi* and Om Prakash, (2007) studied the volatile constituents of *A. allughas*. In both the species the key compound is 1, 8 cineole and β-pinene. The essential oil of rhizome of *A. calcarata* revealed the presence of 1,8 cineole (42%) and was found to be the major constituent in the rhizome oil. The other compounds are fenchone, methyl thymol, α-terpinyl acetate, valencene and elemol (Tewari et al., 1999). Arambewela et al., (2005) reported that the major compound in *A. calcarata* was 1, 8 cineole. Merh et al., (1986) reported the analysis of *A. calcarata* has revealed the presence of protocatechinic acid, quercetin, 4-O-methyl-syringic acid, vanillic acid methyl cinnamate and several terpenes and diterpenes as constituents. 1, 8-cineole had been found to be the major constituent in the oil (Merh et al., 1986; Kong et al., 2000; Tewari et al., 1999; Rath et al., 1994).
The phytochemical study on the whole plant of *P. lanceolata* was carried out by Hendrickson, (1959). Richards & Hendrickson, (1964) isolated $\beta$-sitosterol and propyl 4-hydroxy benzoate from the whole plant of *P. lanceolata*. Dasgupta, (1967) reported $\beta$-sitosterol, acetyl choline chloride and a quaternary base plucheine from *Pluchea lanceolata*. Bahl et al., (1968) reported quercetin and isorhamnetin from *P. lanceolata*. But nobody has studied in detail about the rhizomatous portion of this plant. In the present study hexane extract of these three plants shows marked difference in their Rf values. The TLC plate visualized under UV 254 nm reveals that there are no common bands between these plants and in UV 365 nm after derivatization one band at Rf (0.28) was found to be common for three plants and another one common for *A. calcarata* and *P. lanceolata* at Rf 0.33. All the remaining bands are different for these 3 rhizomes. The same type of bands was observed in visible also. The comparative Gas chromatographic profile of the three rhizomes from the present study also revealed marked difference in their profile and GCMS analysis was carried out in the present study to compare the compounds and identified the major constituents present in these three rhizomes. In the present GCMS analysis we found 14 major compounds in *A. calcarata* and 8 major compounds in *A. galanga* and in *P. lanceolata* 11 major compounds were identified. The market survey of *rasna* was conducted in the present study to find out the extent of variability in the choice of the drug *rasna* in
Kerala. In the present work it is observed that all the three plants are being sold as *rasna* in Kerala market but it is demarcated as North Indian *Aratta (varavaratta)* and *Aratta* and *cittaratta*. The anatomical and phytochemical analysis of these samples revealed that in Kerala market it is calculated that about 90% of the material is *P. lanceolata* and the remaining 10% is *Alpinia* species. The biological activity of *A. galanga* is reported against rheumatism, stomach and bronchial disorder (Perry *et al.*, 1980). The other therapeutic reported characters are tonic, stomachic, carminative, stimulant, aphrodisiac (Nadkarni, 1954; Chopra *et al.*, 1956; Anonymous, 2003). It is used in bronchial catarrh, rheumatism, palpitation of heart and respiratory diseases especially in the case of children (Sarin, 1999). Whereas *A. calcarata* is recommended as aphrodisiac, treatment of bronchitis, cough, respiratory ailments, diabetes, asthma, arthritis (Ramanayake, 1994; Arambewela *et al.*, 2005). Sharma *et al.*, (1980; Jayaweera *et al.*, (1982) and Basnayake *et al.*, (1995) in short the major use of *A. calcarata* for the treatment of rheumatism bronchial catarrh and asthma, blood purification, carminative and arthritis. Kirtikar & Basu, (2006), Anonymous, (1986) and Watt, (1989) reported that *A. calcarata* has the similar therapeutic properties of *A. galanga*. Dwived, (1949) reported that *P. lanceolata* can be used for the treatment of rheumatism, dyspepsia and bronchitis. *P. lanceolata* is also used for the inflammations and bronchitis, cough, psoriasis and piles. The decoction of plant is used to prevent the swelling of...
joints in arthritis and neurological diseases and auto immune disorders (Anonymous, 1969; Billore et al., 2005).

From the above literature survey it is found that these three plants are used for the treatment of rheumatism and bronchitis. *A. galanga* and *A. calcarata* showed almost similar therapeutic properties. Chemical characterization wise *Pluchea* and *Alpinia* stands separately according to the present observations. In Ayurveda, *rasna* is effectively used for the management of patients suffering from rheumatoid arthritis. Sukh Dev, (2006); *Dhanvantari nigantu*, (2005) reported that *rasna* is given in poisonous conditions, rheumatic cases, bronchial disorders and blood disorders. In the case of drug *rasna* though there are no chemical similarities the three plants it is used for the treatment of rheumatism and bronchial disorders. Correlating the antirheumatic activity of these three plants to the therapeutical claim of *rasna* recorded in ancient texts shows the possibility of using these plants as genuine or substitute of each other and further pharmacological studies for other therapeutic activities of these three plants has to be carried out for the better understanding of these plants.