REVIEW OF LITERATURE
2. REVIEW OF LITERATURE

A. 1. General aspects

2. More enquiry on classical and modern approach of selected plants

B. Statement of objective of the present investigation

2.1 General aspects

Pharmacognosy is concerned with the study of crude drugs of vegetable and animal origins. The word “Pharmacognosy” was used by C.A Seydler in 1815 (Greek: Pharmacon = drug; Gnosy = knowledge) (Ali, 2004). Pharmacognosy according to Youngken, (1950), Trease, (1966) and Wallis, (2004) which is an applied science aims at a complete and systematic knowledge of crude drugs of vegetable, animal or mineral origin. It implies not only drugs but also includes a knowledge of sources from which the drugs are prepared, their history, properties and uses, distribution, cultivation, collection and selection. Pharmacognosy is an important link between pharmacology and medicinal chemistry. The knowledge of pharmacology is essential for understanding action of drugs on animals and and the human system. Pharmacognosy is the infrastructure on which depends evolution of novel medicines, as it seen that several crude drugs are utilized for preparation of galenicals or as a sources of therapeutically significant substances that cannot be synthesized economically (Kokate et al., 1999).
Some of the Indian workers have surveyed the Indian Medicinal Plants with respect to their Pharmacognostical characters and the active constituents such as volatile oils, fixed oils, gums, sterols and alkaloids (Aiyar & Kolammal, 1951-1979). Mehra et al., (1969) in his review on researches in Pharmacognosy has described quite a number of drugs along with their proven substitutes and adulterants.

In the standardization of a drug, organoleptical, morphological, anatomical, physicochemical, phytochemical (qualitative and quantitative) and chromatographical methods are used. Morphological and anatomical characters play a vital role in crude drug standardization. Morphological characters involve size, arrangement, venation, texture, surface characters, markings and hardness of the plant materials. As stated by Metcalfe & Chalk, (1957) microscopical methods are often necessary to establish the botanical identity of commercial samples of medicinal plants, timbers, fibers etc. and may play an important part in checking adulteration and substitution. It involves longitudinal and transverse sectional views of the parts of the drug.

Organoleptical characters play an important role in the identification of crude drugs. In this method, the colour, fractures, taste and smell of the drugs are characterized. Chakraborti et al., (1988) studied the stem barks of Strychnos nux-vomica and S. potatorum, and distinguished the authenticity of 'Nux-vomica' bark from other barks.
Shah & Khanna, (1961) distinguish the fruits of *Embelia ribes* with its grayish black colour and warty surfaces with that of *E. robusta*, which has reddish, longitudinally wrinkled surface and more prominent calyx with five sepals.

Physicochemical and phytochemical studies include ash value, solubility and extractive values and qualitative and quantitative analysis of phytochemicals. Satakopan & Thomas, (1970) distinguish the leaves of *Adhatoda vasica* and its adulterant and *Ailanthus excelsa* based on the palisade ratio and anatomical characters of leaf and petiole. *Mucuna cochinchinensis* is the adulterant of the Unani drug 'Karanj' (Genuine: *Pongamia Pinnata*). Hasmi & Singh, (1997) established it with the use of fluorescence analysis and other pharmacognostical parameters.

Quantitative estimation of major phytochemical constituents of a drug is another parameter. Liu *et al.*, (1993) estimate the alkaloid content of three species of *Phellodendron* and distinguish them with one another based on quantity of alkaloid and texture and color of the herbs. Geographical and climatic factors influence the percentage of active constituent of a medicinal plant. Dadun *et al.*, (1992) concluded that the percentage of alkaloids content of *Ephedra sinica* is dependent upon the season and geographical locality in which the plant is grown. Dragur & Menary, (1992) proved that the oil yield of *Olearia phlogopappa* is higher in summer months than in autumn. Mallavarupu *et al.*, (1995) estimate the essential oil content of
Cinnamomum zeylanicum grown in different localities. It was found that the plants grown in Hyderabad are having more oil percentage than that in the plants grown in Bangalore.

Khatoon et al., (1993) used TLC finger printing technique and identify that the market samples 'Ratanjot' is derived from Arnebia nohilis. Asif & Shafiullah, (1993) analyzed 175 herbal drugs with Infrared spectrum and evolved a method for checking the purity of herbal drugs. Mitra et al., (1976) after careful pharmacognostical study stated that no difference was found in the macro and micro characters in rhizome of red and white varieties of Nelumbo nucifera. Abdurahman et al., (1996) tried to distinguish the two varieties of Irringia gabonensis based on their pharmacognostical characters.

Many of the early investigators were contented by describing the pharmacognostic characterization but more enquiry reveals that a thorough phytochemical and pharmacological approaches have also been attempted in medicinal plants. The detailed review of the selected species is collected for the present study.

2.2 Citraka

Citraka, one of the most important drugs in indigenous medicine is recognized on the basis of flower colour. According to Ayurveda there are three varieties of citraka 1) white (sita) 2) red (rakta) and 3) black (Krishna) (the black variety in the classical text may refer to the blue flowered one). These are botanically identified as Plumbago zeylanica, Plumbago indica and Plumbago capensis respectively.
Astangahrdaya, (2000) stated that Asita (means non white) is the best variety. Singh & Chunekar, (1972) has opined that Vaidyas of Bengal take particular care to use only the red flowered species and this may be the Asita variety of Vagbhata. Chunekar, (Bhavaprakasa nigantu, 1982) in his commentary of Bhavaprakasanighantu states that while the white Citraka is seen plently, red is rare to find naturally and is made available by cultivation. Among the two types, the roots of white flowering variety (Plumbago zeylanica) is largely used in Northern parts of India while the red one (Plumbago indica) is mostly procured in Kerala as Citraka. Root of Citraka is officinal part in Ayurveda and this enters into many formulations. It is poisonous and has been recommented for use only after proper purification process.

2.2.1 Distribution

P. indica has distributed throughout India in moist situations as well as cultivated, commonly cultivated in gardens throughout India (Nadkarni, 1954; Warrier et al., 2001; Nambiar et al., 2003; Gupta et al., 2008). P. zeylanica is growing wild in Bengal, UP, Southern India & Ceylon (Nadkarni, 1954).

2.2.2 Taxonomy and Morphology

P. indica is a perennial pretty scandent shrub, stems woody, straight with flexible branches; leaves simple, alternate, exstipulate, entire,
membraneous, wavy, short, cuneate at the base passing in to a very short amplexicaul auriculate, red petiole (Iyer & Kolammal, 1960; Sivarajan & Balachandran, 1994; Warrier et al., 2002). Flowers scarlet or bright red colored in long terminal spikes, bracts and bracteoles ovate, nearly equal in size, much shorter than the calyx. Calyx greenish red, sub-sessile, short cylindric, acutely five-toothed, 5 ribbed (Hooker, 1882; Antony, 1989; Gamble, 1921; Mohanan & Henry, 1994; Sivarajan & Mathew, 1996).

*P. zeylanica* is a perennial sub-scandent under shrub found throughout India, much cultivated, wild in west peninsular India and probably in West Bengal, Malaya, Peninsula, Sri-Lanka and waste lands of sub-arid areas of India (Hooker, 1882; Gamble, 1921; Manilal & Sivarajan, 1982; Mohanan, 1984; Ansari, 1985; Ramachandran, & Nair, 1988; Antony, 1989; Vajravelu, 1990; Mohanan & Henry, 1994; Subramanian, 1995; Sivarajan & Mathew, 1996; Sasidharan, 1998; Sasidharan, 1999; Sunil, 2000; Sharma et al., 2001; Anonymous, 2001; Sasidharan, 2002; Kirtikar & Basu, 2006).

### 2.2.3 Pharmacognosy

Pharmacognosy of *P. zeylanica* was reported by Dutta & Mukerji, (1950), Iyengar & Pendse, (1962) and Karnick, (1982). Whereas Nambiar et al., (2003) reported the Pharmacognosy of *P. indica* and
Iyer & Kolammal, (1960) studied the anatomy of *P. zeylanica* compared with *P. indica*.

### 2.2.4 Phytochemistry

Roots of *Plumbago rosea* Linn. are the richest source of plumbagin. Chemically identified as a naphthoquinone, the compound is claimed to sublime at 90°C. Plumbagin, being hydrophobic and insoluble in water, was precipitated out by addition of water to the acetone extract. The filtered residue was taken in chloroform, and the concentrated chloroform extract, when subjected to column chromatography, yielded plumbagin (1.65%), elution being carried out with n-hexane: ethyl acetate (92:8). The identity of the compound was confirmed by melting point data, UV, IR, and mass spectral data reported in the literature. The purity of the compound was further analyzed by subjecting the compound to HPTLC studies (Kapadia *et al.*, 2005).

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 – tetramethyl ether 1, ampelopsin – 3′, 4′, 5′, 7 – tetramethyl ether 2, plumbagic acid 3 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 were reported by Ariyanathan *et al.*, (2010). The roots of *P. indica* accumulate the naphthoquinone plumbagin (Thomson, 1957).
Sankaram et al., (1976) isolated six pigments 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 now becomes a powerful tool for the quality control of raw plant material. The HPLC estimations of plumbagin in *P. indica* & *P. zeylanica* were reported by 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 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 phenolic fraction of the extract of 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*. They 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). They 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 3-0-β-glucopyranosyl plumbagic acid and 3-0-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.*, (1977). Mallavadhani *et al.*, (2002) reported the characterization of plumbagin. In *P. indica* 6-hydroxy plumbagin, sitosterol, stigmasterol, campesterol, plumbagic acid lactone, two flavonyl methyl ethers- azaleatin, cyanin and two aliphatics- palmitic acid and myricyl palmitate (Dinda *et al.*, 1992; Dinda *et al.*, 1999).
Akella et al., (1976) reported chitranone a new binaphthaquinone from *P. zeylanica*. Gopinath et al., (2009) reported a simple, rapid, precise RP-HPLC method was developed for simultaneous estimation of plumbagin and embelin containing different extract. 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, lupanone, sitosterone and stigmasterol (Akella et al., 1976; Chowdhury et al., 1981; Dinda et al., 1989; 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).

### 2.2.5 Pharmacological activity

**Plumbagin**

Plumbagin, the most active naphthoquinone derived from the species of *Plumbago, Drosera* and *Diospyros*, had been wildly studied on pharmacological activities. In small doses, it is a sudorific and stimulates the central nervous system, while in large doses may cause death from respiratory failure and paralysis. The
pharmacological activities of plumbagin have been reported as follows

**Antitumor activity**

Plumbagin exhibited anticancer activity against melanoma cells line (Bowes cell) and breast cancer cells line (MCF7) with IC₅₀ values of 1.39 and 1.28 μM, respectively (Nguyen et al., 2004).

For breast cancer cells, plumbagin exhibited cell proliferation inhibition by inducing cells to undergo G₂-M arrest and autophagic cell death. Blockade of the cell cycle was associated with increased p21/WAFI expression and Chk2 activation, and reduced amounts of cyclin B₁, cyclin A, Cdc2, and Cdc25C. Plumbagin also reduced Cdc2 function by increasing the association of p21/WAFI/Cdc2 complex and the levels of inactivated phospho-Cdc2 and phospho-Cdc25C by Chk2 activation (Kuo et al., 2006).

Anticancer effect of plumbagin had been reported against human non-small cell lung cancer cells A549 with IC₅₀ value of 11.69 μM. It exhibited effective cell growth inhibition by inducing cancer cells to undergo G₂-M phase arrest and apoptosis. Blockade of cell cycle was associated with increased levels of p21 and reduced amounts of cyclin B₁, Cdc2, and Cdc25C. Plumbagin treatment also enhanced the levels of inactivated phosphorylated Cdc2 and Cdc25C. Blockade of p53 activity by dominant-negative p53 transfection partially decreased plumbagin-induced apoptosis and G₂-M arrest, suggesting it might be operated by p53-dependent and independent pathway.
Plumbagin treatment triggered the mitochondrial apoptotic pathway indicated by a change in Bax/Bcl-2 ratios, resulting in mitochondrial membrane potential loss, cytochrome c release, and caspase-9 activation (Hsu et al., 2006).

**Anti-inflammatory activity**

Plumbagin exhibited an immunomodulatory effects by inhibition of T cell proliferation in response to polyclonal mitogen Concanavalin A (Con A) by blocking cell cycle progression (IC₅₀ value of 50 nM). It also suppressed expression of early and late activation markers CD69 and CD25, respectively in activated T cells. The inhibition of T cell proliferation by plumbagin was accompanied by a decrease in the levels of Con A induced IL-2, IL-4, IL-6 and IFN-7 cytokines (Checker et al., 2009).

**Antimalarial activity**

It has been reported that plumbagin exhibited *anti-Plasmodium falciparum* activity by inhibition of isolated *P. falciparum* enzyme, succinate dehydrogenase (SDH), with IC₅₀ value of 5 mM. It also inhibited *in vitro* growth of *P. falciparum* with IC₅₀ value of 0.27 mM (Paiva et al., 2003).

**Antibacterial activity**

Plumbagin has been reported as an *Anti-Helicobacter pylori* agent with MIC value of 4.0 μg/ml, which more potent than that of metronidazole (MIC value of 32 μg/ml) (Park et al., 2006).
Fair et al., (1985) reported on an antibacterial activity of plumbagin against wild-type *E. coli* strain AB 1157 with 99.9% killed by exposure to 1.0 mM plumbagin for 1 hour at 37°C. Antibacterial mechanism of plumbagin may be due to its toxicity by generated active oxygen species and may damage DNA besides a pathway via H$_2$O$_2$.

In contrast, Jamieson et al., (1994) conducted tests in wild-type strain *Saccharomyces cerevisiae* S150-213 and mutated strains using disruption mutations in the genes encoding of two superoxide dismutases, Cu/ZnSOD (*SOD1*) and mitochondrial MnSOD (*SOD2*), and showed that the *SOD1* mutant was 100-fold more sensitive to plumbagin than its parent, while the sensitivity of the *SOD2* strain to plumbagin was indistinguishable from that of the wild-type strain. Thus, Cu/ZnSOD was the principal superoxide dismutating genes target.

Kamal et al., (1995) conducted *in vivo* anti-*S. aureus* test in female mice and showed that plumbagin was noticed to increase in the activity up to 8 weeks with 25 µg/kg body weight, due to its ability to stimulate the response on oxygen radical release by macrophages. While at high dose (50 µg/kg body weight), it has direct inhibitory activity against *S. aureus*. Antibacterial activity was reported in petroleum ether, chloroform and ethanolic extracts of root but not in the aqueous extract (Vander Vijver & Lotter, 1971).
**Mutagenic activity**

Plumbagin was reported as an antimutagenic activity against *Salmonella typhimurium* TA98 when induced by 2-nitrofluorene (2NF), 3-nitrofluoranthene (3-NFA) and Initropyrene (1-NP) (Edenharder & Tang, 1997). Moreover, for *Escherichia coli* WP2s (uvrA trpE), plumbagin was not mutagenic when presence of plasmid pKM 101 (Kato et al., 1994).

**Antifertility activity**

Plumbagin containing albumin microspheres were implanted to 20 days pregnant albino rats and found that their ovaries showed clear inhibition of growth of graffian follicules and degeneration of the mature follicles, and corpus luteum were observed and result to failed to conceive, the antifertility action of plumbagin seemed to be related to its antiovulatory action (Kini et al., 1997).

**Abortifacient activity**

Plumbagin administered by intubation to albino female rats at 10 mg/kg for 15 days significantly inhibited mating and prolonged duration of estrus cycle and diestrus phase. Plumbagin showed a dose-related abortifacient activity in rats administered 5-20 mg/kg orally from Day 5 to 11 of pregnancy. At doses 10-20 mg/kg from days 1 to 5 of pregnancy, plumbagin caused a significant anti-implantation effect. No gross teratogenic effects were noticed in pups born to female rats that had received 5 or 10 mg/kg plumbagin from
days 1 to 5 of pregnancy (Premakumari et al., 1977). Root powder caused abortions (Kanase, 1995) and preimplantationary loss due to its effect on uterine protein content (Devarshi et al., 1991).

**Reproductive toxicity**

Plumbagin has demonstrated reproductive toxicity in male and female animals. Teratogenic effects were not seen in limited studies. Only one of 12 female Long-Evans rats intubated with plumbagin at 10 mg/kg for 10 days conceived, bearing a litter of five pups. All 12 control animals conceived, producing an average litter size of six pups. One animal in the plumbagin group died of hemorrhage that the authors suspected was caused by competitive inhibition of vitamin K activity, needed for the synthesis of clotting factors (Chowdhury et al., 1982).

Plumbagin given orally at 10 mg/kg for 10 days to adult female rats of the Holtzman strain caused a highly significant decrease in the weight of ovaries as compared with the controls (Santhakumari & Suganthan, 1980).

Plumbagin administered intra-peritoneal at a dose of 10 mg/kg for 60 days caused selective testicular lesions in dogs. The wet weights of testes and epididymides were decreased. In addition, the seminiferous tubule and Leydig cell nuclei diameter were significantly decreased and cellular heights of epididymides were drastically curtailed (Bhargava, 1984).

Oral administration of plumbagin to male gerbils at 10 mg/day for
20 days caused a decrease in the wet weight of seminal vesicle and prostate glands. The cell height of the secretory epithelium was also decreased, and little secretion in the lumen of these glands was observed (Bhargava, 1984).

Plumbagin caused a decrease in the number of spermatids, resting and pachytene spermatocytes, and a significant reduction in seminiferous tubule and Leydig cell nuclei diameter when given orally to immature Wistar rats at 10 mg/kg for 32 days (Bhargava, 1986).

**Cardiotonic action**

Plumbagin produced a triphasic inotropic response in guinea-pig papillary muscle. Plumbagin did not cause any positive inotropy under anoxic conditions, and the positive inotropic effect was markedly inhibited by oxidative phosphorylation uncouplers (Itoigawa et al., 1991).

**Hypolipidemic and antiatherosclerotic effects**

When administered to hyperlipidaemic rabbits, plumbagin reduced serum cholesterol and LDL cholesterol by 53 to 86 percent and 61 to 91 percent, respectively. Furthermore, plumbagin treatment prevented the accumulation of cholesterol and triglycerides in liver and aorta and regress atheromatous plaques of the thoracic and abdominal aortas (Sharma et al., 1991). Ethanolic extract showed hyperglycaemic effect (Olagunju et al., 1999; Olagunju et al., 2000;),
while 50 per cent ethanolic extract showed central nervous system stimulatory action in rats (Bopaiah & Pradhan, 2001).

**Effects on microsomal enzymes**

Plumbagin exhibited a potent, dose dependent inhibitory activity against aromatase cytochrome P450 in human placental microsomes. However, plumbagin showed relatively weak reducing effects in the presence of microsomal membranes suggesting that the inhibitory effects on monooxygenase reaction were not due to the formation of superoxide radicals (Muto et al., 1987).

Plumbagin in *P. indica* possessing various pharmacological activities like Antimalarial (Likhitwitayawuid et al., 1998), antimicrobial (Didry et al., 1994) and cardiotonic activity (Itoigawa et al., 1991).

Plumbagin in *P. indica* roots exhibits antimicrobial (Didry et al., 1994), insecticidal (Magdum et al., 2001), antitumour (Fujii et al., 1992; Sugie et al., 1998) and antifertility activies (Kini et al., 1997).

Plumbagin is the major constituent of the root of *P. zeylanica* and reported to possess antimicrobial, antiprotozoal, antifertility, pesticidal activities (Devarshi et al., 1991).

**Antifertility activity**

Antitumor and antifertility activities of plumbagin have been reported by Kini et al., (1997). Savadi et al., (2009) reported the ethanolic extract of roots of *P. indica* was evaluated for antifertility activity using antiimplantation, abortifacient and motility of rat spermatozoa
(in-vitro) models. The anti-implantation effect seems to be depending on the dose as well as the initiation of treatment on specific days of pregnancy. *P. indica* has showed percentage pre-implantation loss of 40% and 50% against control at the doses of 200 and 400 mg/kg b/w. Santhakumari & Sugathan, (1980) observed the antigonadrotrophic activity of plumbagin isolated from the roots of *P. rosea*.

Acetone extract of *P. indica* stems exhibited activity in interrupting the normal estrous cycle of female Albino rats at two dose levels, 200 and 400 mg/kg. The rats exhibited prolonged diestrous stage of the estrous cycle with consequent temporary inhibition of ovulation. The anti-ovulatory activity was reversible on withdrawal of the extract. The effective acetone extract was further studied on estrogenic functionality in rats. The acetone extract showed significant estrogenic and antiestrogenic activity. Histological studies of the uteri further confirmed the estrogenic activity of the acetone extract (Sheeja et al., 2009).

Chowdhury et al., (1981) reported that apart from plumbagin, the glycosides and tannin (plumbagin free alcoholic extract) present in the alcoholic extract could be the active principles possessing anti-fertility activity. Saxena et al., (1970) screened out *P. zeylanica* as an antifertility plant on the basis of their study. The antifertility effect has also been reported by Agrawal & Arora, (1972). Plumbagin complexed with hydroxy propyl betacyclodextrin showed antifertility

**Antiparasite activity**

*P. indica* roots extract showed a macrofilaricidal property against *Setaria digitata*, a filarial parasite of cattle. Complete inhibition of motility was observed at concentrations range between 0.02 and 0.05 mg/ml. Fractionation of the crude extract resulted in the isolation of the active molecule plumbagin (Paiva et al., 2003).

**Antimicrobial activities**

Ethanolic extract of *Plumbago zeylanica* root was investigated for its antimicrobial activities against 11 human pathogenic bacteria and 6 phytopathogenic fungi using disc diffusion method and poisoned food technique respectively. The extract exhibited good antibacterial and antifungal activities against the test organisms. The root extract from *P. zeylanica* seems promising since it showed both antibacterial and antifungal activities (Rahman & Anwar, 2007). *P. zeylanica* is used for body aches (Tirkey Amia, 2004). Murty et al., (1998) investigated the anti-oxidant nature of plumbagin against lipid peroxidation induced by oxidants. It indicated that 1 mM concentration of plumbagin prevented the oxidative stress, induced lipid peroxidation in liver and heart homogenates of rats.

Chakraborty et al., (1997) reported *Plumbago zeylanica* possesses antimicrobial activities. Roots and aerial parts principally contain an
orange yellow pigment, plumbagin, a naphthoquinone and a fatty alcohol. Its other constituents in roots are chitranone, zeylanone, dihydrosterolone, 2-methyl naphthaquin, plumbazeylanone and terpenoids, lupeol and teraxesterol. The plant also contains alkaloids, glycosides, tannin, saponins and steroids. Pharmacological screening of _Plumbago zeylanica_ has revealed that the alcoholic extract possesses antimicrobial activity (Ahmad _et al._, 1998). Alcoholic and aqueous crude extracts showed anticandiclal activity and antifungal activity against dermatophytes (Mehmood _et al._, 1999).

**Anticarcinogenic activity**

Plumbagin, isolated from the plant _Plumbago rosea_ is reported to have anti-cancerous property. In order to evaluate the potential of iodinated plumbagin for tumor therapy, plumbagin was radioiodinated with $^{125}$I and evaluated in tumor bearing mice (Ketaki _et al._, 2004). The aqueous and alcoholic extracts of root of _P. indica_ showed abortifacient activity in rats (Prakash & Mathur, 1976). Plumbagin, a plant- derived naphthoquinone, has been shown to exert anticarcinogenic and anti-atherosclerosis effects in animals. Plumbagin inhibited the activity of Nox-4 in a time- and dose-dependent manner in HEK293 and LN229 cells. Plumbagin inhibited the superoxide production in Nox-4 transfected COS-7 cells. These results indicated that plumbagin directly interacted with Nox-4 and
inhibited its activity (Yaxian et al., 2005).

Jai et al., (2004) reported that the active constituent Plumbagin of this plant acts as antioxidant. They found that the hydroxyl (OH), Alkyl peroxy (C\textsubscript{C}I\textsubscript{3}OO), Linoleic acid peroxy (LOO) and Glutathiyl (GS) radicals generate a phenoxyl radical upon reaction with plumbagin.

The tumour inhibitory and radiomodifying effects of plumbagin, a naphthoquinone isolated from Plumbago rosea (P. indica), on mouse Ehrlich ascites carcinoma was studied by Devi et al., (1999). Plumbago zeylanica is used against stomach trouble in domestic animals by tribal and non tribal rural people (Sikwar, 1994); Plumbago zeylanica is used for the treatment of jaundice (Singh et al., 2004). Yuan-Chuen et al., (2005) reported Plumbago zeylanica L. had the highest inhibitory effects against \textit{H. pylori}.

Premlata et al., (1993), demonstrated that the active constituent Plumbagin, isolated from Plumbago zeylanica Linn., when administered orally, at a dosage of 4 mg/kg body weight induces tumour regression in 3-methyl-4-dimethylaminoazobenzene (3 MeDAB) induced hepatoma in Wistar male rats. The purpose of their investigation was to identify the changes in the rate of glycolysis and gluconeogenesis in tumour-bearing rats and the effects of treatment with Plumbagin. They observed that the levels of certain glycolytic enzymes, namely, hexokinase; phosphoglucoisomerase; and aldolase levels increased (p<0.001) in hepatoma bearing rats, whereas they
decreased in plumbagin administered rats to near normal levels. Their investigations indicate the molecular basis of the different biological behaviour of 3MeDAB induced hepatoma and the anticarcinogenic property of Plumbagin against hepatoma studied in rats.

**Wound healing activity**

The ethanolic extracts of *P. zeylanica* have wound healing activity in rats (Suresh Reddy *et al.*, 2002). *In vitro* antibacterial and anti fungal activities of the crude extract of the plant were determined by disc diffusion method (Bauer *et al.*, 1966) and poisoned food technique (Miah *et al.*, 1990). Mueller-Hinton medium (agar and broth) was used for culture for bacteria and Sabouraud medium (agar and broth) was used for culture of fungi, ethanolic solution (5%) of the crude extract was used as the test antimicrobial agent against 11 human pathogenic bacteria and 6 phytopathogenic fungi. The results were compared with the standard antibacterial, antibiotic ampicillin (20µg/disc, Beximco Pharma Bangladesh Ltd., Dhaka) and antifungal antibiotic nystatin (100µg/ml medium, Beximco Pharma Bangladesh Ltd., Dhaka). Minimum inhibitory concentration (MIC) of the crude extract was determined by macro-dilution broth technique (Johnes *et al.*, 1985).

*Plumbago zeylanica* in a dose; 10 mg/kg b.wt., ip, for 60 days caused selective testicular lesions in dogs (Bhargava, 1984). Plumbagin, isolated from roots *P. zeylanica*, was found to prolong estrous cycle
and induce anti-implantation effects (Premkumari et al., 1977). In addition, plumbagin is also shown to have a glucogenic effect (Parimala et al., 1993; Olagunju et al., 1999). Bhargava, (1984) studied the effect of plumbagin, isolated from the roots of Plumbago zeylanica on reproductive function of male dog and highlighted the influence of plumbagin on the testicular and epididymal function of dog and injected (ip) plumbagin at a dose of 10 mg/kg b.wt. on alternate days for a period of 60 days and concluded that plumbagin inhibits spermatogenesis as well as it alters epididymal function.

**Antifungal activity**

Hydroalcoholic (80% ethanol) extract of *P. indica* root possessed potent antifungal activity against *Aspergillus niger* and *Candida albicans* (Valsaraj et al., 1997). In addition, plumbagin had been reported as the active compound against *C. albicans* with MIC and MFC (Minimum fungicidal concentration) values of 0.78 and 1.56 μg/ml, respectively (Figueiredo et al., 2003).

Ahmad et al., (1999) screened out the alcoholic and aqueous crude extracts of 37 traditionally-used medicinal plants which included *P. zeylanica* for antifungal activity. They selected alcoholic extracts of five medicinal plants for further studies on the basis of their strong anticandidal activity. They observed that the Minimum inhibitory concentration (MIC) values ranged between 4 - 9 mg/ml. Among the five they observed comparatively high potency in the extracts of *P. zeylanica* (4mg/ml). They hypothesized that the synergistic action of
these plant constituents may be responsible for enhanced activity in these plant extracts, especially in *P. zeylanica* which showed high potency.

According to Krishnaswamy & Purushothaman, (1980) Plumbagin, isolated from *Plumbago zeylanica*, showed positive results when given intratumor and orally at 10 µg/ml induced fibrosarcoma in wistar strains of rats.

**Anti-inflammatory activity**

Oyedapo, (1996) investigated the anti-inflammatory activity of the phosphate buffered saline extract of the roots of *P. zeylanica*. They observed that the extract stabilized red blood cells subjected to both heat and hypotonic induced lyses and the extract exhibited a biphasic response.

**Antibacterial activity**

Hydroalcoholic (80% ethanol) extract of *P. indica* roots exhibited antibacterial activity against *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Escherichia coli* and *S. aureus* (Valsaraj *et al.*, 1997). Moreover, plumbagin had been reported as the active compound against *S. aureus* with MIC and MBC values of 1.56 and 25.0 µg/ml, respectively (Figueiredo *et al.*, 2003).

Lemma *et al.*, (2002) carried out the antibacterial activity of the plant. They adopted the ‘Hole plate diffusion’ method. They found that the petroleum ether extract of the root of *P. zeylanica* possesses
strong activity against some pneumonia causing pathogens. They further identified the active compounds as 5-hydroxy-2-methyl-1,4-naphthoquinone and plumbagin.

Antibacterial activity of plumbagin and of methanol, chloroform extracts of *P. zeylanica* L. root against various pathogenic bacteria and the minimum inhibitory concentrations. Plumbagin and chloroform extracts of *P. zeylanica* root showed antibacterial activity against *Escherichia coli* *Salmonella typhi*, *Staphylococcus aureus*. The methanolic extract exhibited moderate activity and the aqueous extract weak activity against the bacterial strains as assessed by disc diffusion assays. The bioactive compound plumbagin and extract of *P. zeylanica* root show a wide spectrum of antibacterial activity. The compound shows promise as a new drug for various bacterial infectious diseases (Jeyachandran *et al.*, 2009).

Wang & Haung, (2005) studied the antibacterial activity of the root extract of *P. zeylanica*. They observed that the plumbagin was active against *Helicobacter pylori* even in low concentration as the minimum inhibitory concentration was 0.02-0.16mg/ml and 0.16-1.28mg/ml as minimum bacterial concentrations. They observed the bactericidal activity over a wide range of pH 2-7 and good stability over the range of pH 1-7.

Krishnaswamy & Purushothaman, (1980) also reported the same property of *P. zeylanica*. In his study, they tested the antibacterial activity of plumbagin against *Staphylococcus citreus*, *S. aureus*, *S.*
albus, *Salmonella paratyphi*, *S. dublin*, *Corynebacterium equi* and *Klebsiella pneumoniae* at 20μg/ml.

**CNS activity**

Springob *et al.*, (2007) reported that *P. indica* contains naphthoquinones that are derived from six acetate units. cDNA encoding a type III polyketide synthase (PKS) was isolated from roots of *P. indica*.

Bopaiah & Pradhan, (2001) investigated the CNS stimulatory action of the root extract of the *Plumbago zeylanica* on rats. They studied the effect of the 50% ethanol extract of the root on locomotor behaviour and central dopaminergic activity in rats. They observed that the ethanolic extract specifically enhanced the spontaneous ambulatory activity without inducing stereotypic behavior. The effect of the root extract showed an inverse relationship between motility and the levels of DA (Dopamine). The CNS activity has also been reported by (Kirtikar & Basu, 2006).

**Antimutagenic activity**

Petroleum ether extract of *P. indica* root showed antimutagenic activity (Rojanapo *et al.*, 1990). The 95% ethanolic extract showed weak antitumour effect, but when used in combination with radiation it showed synergistic effect in inhibiting tumour growth in animals (Devi *et al.*, 1994). Aqueous root extract inhibited
spontaneous and prostaglandin evoked uterine activity of isolated rat uterus (Lal et al., 1983).

**Antimalarial activity**

Methanolic extract of *P. indica* showed antimalarial activity against *Plasmodium falciparum* (Ayudhaya et al., 1987). Various extracts of root showed antibacterial, antimycobacterial and antiyeast activities (Pongapan et al., 1982).

**Antitumour activity**

Plumbagin from *P. indica* root showed antiprogestational (Dhar & Rao, 1995). Chronic treatment with plumbagin caused testicular lesions in dogs (Bhargava, 1984). Plumbagin from *P. indica* showed antitumour activity in rats (Krishnaswamy and Purushothaman, 1980) and mice (Kavimani et al., 1996; Devi et al., 1999). It caused *in vitro* arrest of cell growth and proliferation of chick embryo fibroblasts (Santhakumari & Sugathan, 1980). Plumbagin from *Plumbago* species treatment brought about a significant decrease in cell count of mouse melanoma cells *in vitro* (Prasad et al., 1996) and also enhanced the growth inhibitory effect of gamma radiation, suggesting a radiosensitizing effect of plumbagin (Prasad et al., 1996; Devi et al., 1999). Plumbagin from *P. indica* in combination with gamma radiation produced G2-block, but more pronounced effect was on reducing the labelled 5-phase cells (Devi et al., 1998). It was found to be less toxic to normal bone marrow cells when compared to cyclophosphamide. However, it enhanced the radiation induced cell
lethality (Ganasoundari et al., 1997). Plumbagin exhibited in vitro agglutination of human RBCs and formation of methhaemoglobin (Gopal & Purushothaman, 1986) and anticoagulant activity in rats (Santhakumari et al., 1978).

**Other activities**

Root powder also showed digestive and appetizing property by normalizing the intestinal flora in mice (Iyengar & Pendse, 1966). Crude and ethanolic extract also showed metabolic effects in rat liver (Oyedapo & Amos, 1997). It also possessed radiomodifying properties (Umadevi et al., 1999).

Prajapati et al., (2003) reported the bioactivity of the chloroform extract of *P. zeylanica* against various life stages of a noxious lepidopteran insect-pest, *Spilarctia oblique* Walker showing strong feeding deterrence, growth inhibition and ovicidal effects of the chloroform extract against the eggs and larvae and strong morphogenetic disorders in the pupae.

Oyedapo & Amos, (1997) investigated the bioactivity of crude and ethanol extract of the root of *P. zeylanica* in rats. They reported the damage of hepatic cells of rats, thereby resulting in moderate to high increase in the specific activities of serum LDH, acid phosphatase, liver alkaline phosphatase as well as blood sugars. Oyedapo, (1996) and Thomas, (1989) reported that the root and leaf extracts of this plant possess antimalarial, antimicrobial, anti-inflammatory and antifungal activities.
Pharmacological screening of various extracts of plumbago species revealed that plumbagin is the bioactive compound responsible for the various medicinal activities of the plant, Plumbagin at low doses has a stimulant action on central nervous system, muscles and secretion of sweat, bile and urine (Anonymous, 2003). The root of *P. zeylanica* and its constituents are credited with potential therapeutic properties. The isolation and spectral data for new flavonoid 2-(2, 4-Dihydroxy-phenyl)-3, 6, 8- trihydroxy-chromen-4-one from the roots of *Plumbago zeylanica* were determined and the antioxidant activity were studied by free radical scavenging and superoxide radical scavenging assays (Nile et al., 2010). The effects of 50% ethanol extract of root of *P. zeylanica* were investigated on locomotor behavior and central dopaminergic activity in rats (Bopaiah & Pradan, 2001).

**2.2.6 Therapeutic uses**

The root of *Plumbago indica* is acrid, astringent, thermogenic, constipating, expectorant, gastric, nervous stimulant and rejuvenating. It is useful in dyspepsia, cough, bronchitis, helminthiasis, elephantiasis, intermittent fever, scabies and vitiated conditions of *vata* and *kapha* (Warrier et al., 2002). The root is considered abortifacient, vesicant, and a powerful sialogogue. In Malaya, chewing the roots regularly with arecanut is said to cause abortion. Because of its vesicating properties it has been used in the treatment of certain types of leucoderma. In South India, it is valued as a remedy for secondary syphilis and leprosy. It has been
recommended as an efficient substitute of cantharides. The bruised root is acrid and stimulating. In large doses, it acts as an acronarcotic poison. A tincture of the root is used in dyspepsia, piles, flatulence, loss of appetite and other digestive complaints. In Java, the root is used as a veterinary medicine for expelling worms in horses (Anonymous, 2003). *P.zeylanica* whole plant and its roots have been used as a folk medicine for the treatment of rheumatic pain, dysmenorrhea, carbuncles, and contusion of the extremities, ulcers and elimination of intestinal parasites (Lin et al., 2003). In eastern Africa and India *P. indica* was traditionally used for gastric stimulant, abortifacient and oral contraceptive. An infusion of root is taken to treat dyspepsia, colic, cough and bronchitis. A liniment made from bruised root mixed with a little vegetable oil was used as a rubefacient to treat rheumatism and headache (Schmelzer & Gurib-Fakim, 2008)

Roots of *P.zeylanica* have potential therapeutic properties like anti-anthrogenic, carditonic, hepatoprotective, neuroprotective properties (Tilak, et al., 2004). It has been also reported that the plant have anticancer, antibacterial, antifungal and antitumor properties (Kavimani, et al., 1996); The leaves and roots of *P.zeylanica* contains an alkaloid called plumbagin (2-methoxy-5-hydroxy-1, 4-napthoquinone), which externally is a strong irritant but a powerful germicide; stimulates muscular tissue in smaller doses and paralyzes in larger ones; stimulates the contraction of the muscular tissues of
the heart and intestines; stimulates the secretion of sweat, urine and bile; and also has a stimulant action on the nervous system (Chopra et al., 1996).

The pharmaceutical studies and therapeutic uses of *Plumbago zeylanica*, in fresh root and as well as dry drug (root), were reported (Chetty et al., 2006). The root of *Plumbago zeylanica* in combination with other ingredients is prescribed for epilepsy, hysteria, nervous affections, obesity, indolent ulcers and influenza (Kaushik & Dhiman, 1999). The root of *Plumbago zeylanica* is a constituent of a medicine for flatulence in the form of a powder called *shaddharana yoga* recommended by Susruta. Root is generally used as astimulant adjunt to other preparation in the form of a combination called *trimada*. For chronic and muscular rheumatism and all painful affections of joints, pills or powder called *Chitrakanthi* is recommended (Nadkarni, 1954). *Plumbago zeylanica* is used as ingredient of drug formulations, prescribed in anasarca, billiousness, cough, dyspepsia as an expellant of phlegmatic tumours and prurigo (Sarin, 1999). The root appears to possess abortifacient and vesicant properties. It is diuretic, caustic and expellent of phlegmatic tumours, and is useful in rheumatism. It is used as an irritant to the skin, in the treatment of dyspepsia, piles, anasarca, diarrhoea and skin diseases. In Africa, a cold infusion of the root is used for influenza and black water fever. A tincture of root bark is antiperiodic (Anonymous, 2003).
2.3 Jivanti

Jivanti and its synonyms jivani, jiva, jivada, jivaniya denotes the property of the drug to provide or enhance the vitality of the consumer. In Ayurveda it is a valuable drug being mentioned in Jivaniyagana (group of vitality promoting drugs) and as a pot herb par excellence. Ayurvedic lexicons are of different opinion regarding its varieties. Nighantusamgraaha has mentioned 6 varieties of Jivanti. Abhidhanamanjari has mentioned 2 varieties and Rajanighantu 3 varieties. The drug is an ingredient of many Ayurvedic formulations. But in practice any discrimination or varieties is not recognized. The name Dodi as a popular name has been attributed to Jivanti in Dalhanas commentary on Susrutasamhita (Dalhana, 1982). Jivanti, as it is known today is a controversial drug. Most of the Ayurvedic experts consider Leptadenia reticulata as the source of Jivanti. The Orchid Dendrobium macraei and Holostemma annulare have also been mentioned in their books. Rarely, the names Dregia volubilis and Cimicifuga foetida have also been mentioned but it seems have not gained popularity. KC Chunekar, (Bhavaprakasa nigantu, 1982) in his commentary of Bhavaprakasanigntatu has described Dendrobium macraei along with Leptadenia reticulata. Singh & Chunekar, (1972) seems to be in favour of the Dendrobium macraei more than the other. He shows hope that since the orchids have captured the market for long as some of the most valuable drugs (like the jivaniyagana, Asthavrga etc.) it may receive proper attention
in the hands of modern investigators. Vaidya, (1982) has given impressive reasons for choosing *L. reticulata*. It is known in Gujarath as Dodi and reported to be used as a leafy vegetable. Since *Jivanti* is an excellent pot herb and a member of Orchidaceae might not be a favourable candidate. In his book *Some controversial drugs in Indian medicine* he has quoted the opinion of Acarya Trikamji as well who counts *L. reticulata* having milky latex as *Jivanti* and *D. macraei* having yellow latex as the *Svarnajivanti*. In Kerala, the root of *Holostemma ada-kodien* is procured as *Jivanti* for medicinal purposes since the Keralite commentators and lexicons have favoured Adapathiyan, identified to be the same. In Ayurvedic Pharmacopoeia of India *Leptadenia reticulata* has been suggested as *Jivanti*. Solving this controversy may require a very thorough scientific investigation of each and every plant concerned.

### 2.3.1 Distribution

*Holostemma ada-kodien* has been recorded as occurring in the tropical Himalayas, and W. Peninsula, Konkan, Bombay, Carnatic and Kerala (Santapau & Henry, 1973). It grows over hedges and in open forests especially on the lower slopes of the hills (Kolammal, 1979; Sivarajan & Balachandran, 1994). *Leptadenia reticulata* is found in N. Circars, Deccan & Carnatic, West ward to the E. slopes of the Ghats, up to 3,000 ft chiefly in hedges and also distributed in Mauritius, Madagascar, Sri Lanka, tropical Himalaya, Burma (Sivarajan & Balachandran, 1994; Anonymous, 2008). In India, it is
distributed from Punjab Southwards to W. Peninsula (Biswa, 1955; Santapau & Henry, 1973).

2.3.2 Taxonomy and Morphology

_Holostemma ada-kodien_ is a twining undershrub; leaves opposite membraneous; flowers fleshy, pinkish red, attractive in axillary, few flowers, lax cymes. In recent past, this wild medicinal plant species was recommended for cultivation for remunerative purposes. The package of cultivation practices under processing techniques has been standardized (Hooker, 1883; Gamble, 1923; Manilal & Sivarajan, 1982; Mohanan, 1984; Babu, 1990; Vajravelu, 1990; Nayar, 1992; Mohanan & Henry, 1994; Sasidharan & Sivarajan, 1996; Sasidharan, 1998; Sasidharan, 2002; Mohanan & Sivadasan, 2002). _L. reticulata_ is a twining shrub with well developed leaves; flowers small, in lateral umbellate cyme, having chromosome number 22, occur in tropical Africa and Asia (Hooker, 1883; Gamble, 1923; Babu, 1990; Vajravelu, 1990; Subramanian, 1995; Sasidharan, 1999). Singh, (1943) studied the intra and interxylary phloem in stem; and (Rao & Malviya, 1966), the laticifers in the plant. While studying some market drugs supplied from Haridwar and Dehra Dun areas, _Leptadenia reticulata_ was sold as ‘Jivanti’ reported by Uniyal et al., (1979).

2.3.3 Pharmacognosy

Pharmacognosy of _H. ada-kodien_ was reported by Kolammal, (1979)

### 2.3.4 Phytochemistry

Presence of α-amyrin, lupeol, β sitosterol, alanine, aspartic acid, glycine, serine, threonine and valine is reported from the ethanolic extract of roots *H. ada-kodien* (Ramiah et al., 1981). Unnikrishnan, (1995) reported that the consumption of ‘kashaya’ prepared from *H. annulare* improved the voice in cases of Swarasuda disease (where the voice is lost or becomes defective due to loud talking). 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 by Karmarkar et al., (2001). Generally reports on *H. ada-kodien* are meager.

*Lepeadeina reticulata* has gained attention to some extent from chemical point of view. In a preliminary phytochemical investigation of the species by 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, however, the hentriacontanol, amyrins, stigmasterols and two flavonoids were isolated (Krishna et al., 1975) and in the follicle, β-sitosterol, quercetin and its glycoside (Subramanian, 1968; and Subrarnanian & 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-saponifiables. Stigmasterol and tocopherols were isolated in pure form and identified. *Lepeadenia 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).

A pentacyclic triterpenoid lupeol was isolated from the chloroform extract of the root of *Leptadenia reticulata* (Asclepiadaceae). The structure of the compound was determined by spectroscopic analysis (Rajanna et al., 2009). *L. reticulata* contains leptadenol, hentiacontanol, acetyl alcohol, β-sitosterol, β-amyrin acetate, lupanol 3-O-diglucoside, leptidin, quercetin, iso-quercetin, rutin, hyperoside, simiarenol (3-β-hydroxy-E: B-friedehop-5-ene) a rare triterpenoid alcohol, novel pregnane glycosides namely reticulin, calogenin, denticulatin, and 0.5% alkaloids (Verma et al., 1962; Subramanian & Nair, 1968; Krishna et al., 1975; Subramanian & Lakshmanan, 1977; Srivastava et al., 1994). A fructosan of the inulin type has been separated from the tubers (Arnon, 1949). A considerable amount of sterols is present in the free condition; stigmasterol is the major component and small quantities of sitosterols, of which γ-sitosterol has been identified, are also present. A fructosan (7-8 hexose units) of the inulin type has been separated from the tubers (Anonymous, 1988). *L. reticulata* contains alkaloids, carbohydrates, steroids,
glycosides and reducing sugars (Harbone, 1973; Wagner et al., 1984).

2.3.5 Pharmacological Activity

General activity

In *L. reticulata* the antifertility and prolonged hypotensive activities of the aqueous extract of stem in anaesthetised dogs have been reported by Agarwal *et al.*, (1960); Basu *et al.*, (1961); Patel *et al.*, (1986). A mixture consisting of the extract of this plant and *Breynia patens* Benth. has lactogenic properties (Anonymous, 1972).

*Holostemma ada-kodien*

Antidiabetic activity

Shirwaikar *et al.*, (2007) reported the antidiabetic potential of the alcohol root extract of *Holostemma annulare* Schum. He was evaluated in the streptozotocin-nicotinamide-induced type 2 diabetic model. Graded doses of the alcohol root extract were administered to normal and experimental diabetic rats for 15 days. A significant (p<0.05) reduction was observed in the fasting blood glucose levels of normal as well as diabetic rats. Serum insulin levels were stimulated in the diabetic animals treated with the extract. Body weight, serum lipid profiles, serum urea and creatinine levels were estimated in extract-treated normal and diabetic rats. In addition, glycosylated hemoglobin and liver glycogen levels assessed in the extract-treated diabetic rats were compared with diabetic control and normal
animals. Significant results were observed in the estimated parameters, thereby justifying the use of the plant in the treatment of diabetes mellitus.

The possibility of the antidiabetic effect of *Holostemma annularis* K. Schum (Asclepiadaceae) (HA) in diabetic C57/BL6J *ob/ob* mice; which was considered a good model for type 2 diabetes as it displays many of the characteristics of the human diseases including hyperglycemia, insulin resistance and progressive obesity (Hummel *et al.*, 1966). The roots of *Holostemma annularis*, which is reported to have a potent antioxidant property (Upadhye *et al.*, 2007). β-sitosterol, lupeol, and alpha-amyrin as the main constituents from the roots are reported to have both antioxidant and antidiabetic properties (Sudhahar *et al.*, 2006; Nirmala *et al.*, 2008). The roots of this plant are also reported to have an antidiabetic effect in streptozotocin-induced diabetic rats (Shirwaikar *et al.*, 2007).

The roots of *Holostemma annularis* are used in traditional medicine to treat diabetes. This medicinal plant, widely used in more than 34 ayurvedic preparations, was evaluated in a high fructose diet in induced insulin resistance and in C57BL/6J *ob/ob* diabetic mice for its antidiabetic activity. Graded doses of both chloroform and methanolic extracts of the roots of *H. annularis* were administered to normal and experimental diabetic rats for 21 days. Serum glucose, triglycerides, cholesterol levels and total protein in urine were analyzed and positive results were observed (Reddy *et al.*, 2010).
Further a clinical trial was performed to assess the efficacy of this drug as a galactagogue in lactation. The study revealed that out of 90 cases, 41 cases showed a good response, so much so, that no top feed was required for supplementation. Thirty nine cases showed fair response and 5 cases showed no response (Taly & Gupta, 1992).

**Antioxidant activity**

The alcoholic root extract of *Holostemma annularis* has been shown to have a potential antioxidant activity in both *in vitro* and *in vivo* models (Upadhye *et al.*, 2007).

**Chemoprotective activity**

The *Holostemma annularis* root has been reported to have chemoprotective activity in cyclophosphamide-induced toxicity (Sudhahar *et al.*, 2006). Many studies have validated the role of β-sitosterol and lupeol in the management of diabetes and hypercholesterolemia (Jayaprakasha *et al.*, 2007; Nirmala *et al.*, 2008). β-sitosterol has been shown to normalize blood sugar and insulin levels in type 2 diabetics. The mechanism for this effect is that β-sitosterol stimulates the release of insulin in the presence of non-stimulatory glucose concentrations (Jelly *et al.*, 2006) and inhibits glucose-6-phosphatase (Ivorra *et al.*, 1990). Lupeol present in the roots of *Holostemma annularis* has the ability to protect cells and tissues from oxidative stress, which induces the formation of
cytoprotective enzymes like catalase and superoxide dismutase (Miettinen et al., 1989).

**Leptadenia reticulata**

**Abortifacient activity**

*L. reticulata* also reported to be useful in habitual abortion in women (Anonymous, 1988).

**Antibacterial activity**

The alcoholic extract (50%) of root and leaves of *L. reticulata* exhibited antibacterial activity (Patel & Dantwala, 1958). Agarwal et al., (1960) carried out some antibacterial activity of phenolic and nonphenolic fractions of this plant against 18 organisms. Herbinol, a herbal antiseptic cream which includes *L. reticulata* as an ingredient showed significant anti-microbial activity against some common microbes causing septicaemia (Pandya et al., 1989).

Lupeol from the roots of *Leptadenia reticulata* showed a significant antibacterial and antifungal activity. Antibacterial activity of crude chloroform extract of root of *L. reticulata* has displayed significant to moderate and dose dependent antibacterial activity. The extract of root is active against *E. coli*, *S. aureus*, *P. aeruginosa* and *V. cholerae*. The significant zone of inhibition at 300 μg/well recorded against *V. cholerae*. The isolated pure compound (lupeol) had shown significant antibacterial activity especially against *P. aeruginosa*, *S. aureus* and *V. cholerae* at the concentration of 300 μg/well. While, at 200 μg/well, *E. coli* showed good activity (Rajanna et al., 2009).
**Lactogenetic activity**

Anjaria, (1980) studied the effect of this on lactogenesis in rats and also on their reproductive and endocrine organs when treated with an ayurvedic preparation containing *Aloe indica, Balsamodendron myrrha, Breynia patens, Rubia cordifolia, Pegamnum harmala, Loha bhasma* and *Leptadenia reticulata*. Thirteen mares from Bhopal, Madhya Pradesh having irregularities of oestrous cycle showed successful results. The average duration of effective treatment was 35 to 55 days and the conception rate was 53.85% (Kudale & Amarnath, 1980).

Leptaden, a herbal Ayurvedic drug consisting of *Breynia patens* and *L. reticulata*, is considered to be a good lactagogue. The galactopoietic, lactogenic and galactokinetic actions of this drug were studied in puerperal women as well as in lactating rats. In case of human, no appreciable gain in weight in the babies of treated mother (2 tablets t.i.d. for 7 days followed by 2 b.i.d. for 14 days) was observed whereas marked improvement in the flow of milk in treated rats was noticed (Lal et al., 1980). But Patel et al., (1982) reported that double-blind clinical trial on mothers having deficient lactation exhibited that this non-hormonal ayurvedic drug is a good lactagogue and recommended as safe to the mother as well as to the child who breast is fed under Leptaden therapy.

Role of Leptaden during pregnancy was also studied by Savithri &
Rao, (1981). The response showed that out of 40 cases of habitual abortions treated with Leptaden (2 tablets), 33 cases (82.5%) had FTLB, 4 cases (10%) had premature delivery and 3 cases (7.5%) had abortion. The results indicated that Leptaden is a highly efficacious drug in cases of habitual abortions.

A number of studies have been carried out on the galactagogue property of L. reticulata in laboratory animals. Studies on the lactogenic property of L. reticulata were carried out on lactating rats using pup weight, body weight of mother rats, and histopathological study of lactating mammary gland, as well as the secretory rating, parenchyma percentage, estimation of glycogen content (of the abdominal mammary glands) and the protein content (of the pectoral mammary glands) as parameters. While both stigmasterol and the ether fraction of L. reticulata showed lactogenic effect, as assessed by all these parameters, stigmasterol was found to be more potent (Anjaria et al., 1975). A herbal preparation with L. reticulata as one of the nine constituents was said to exert beneficial effects on the gametogenic and androgenic functions of the testes of animals. It showed anabolic cum androgen like activity as evidenced by the dose related growth of the ventral prostate and the systemic increase in the weights as well as the secretions of the accessory sex organs of castrated adult mice (Jaytilak et al., 1976).

In L. reticulata lactogenic effect of stigmasterol and ether fraction isolated from Leptadenia reticulata were studied on lactating rats. On
consideration of results on parameters of pup weight, body weights of mother rats, photomicrographic studies of lactating mammary glands on the 23rd day, secretory rating, parenchyma percentage, glycogen contents and protein contents of mammary glands, it was observed that both the principles had lactogenic effect on lactating rats (Anjaria et al., 1975). Crude Leptadenia reticulata administered to five cows in the dose of 1.5gm/cow/b.i.d for 15 days produced an increase in milk yield in four out of five cows with a net gain of 10.5%. Clinical study of Leptaden tablets (2 tablets 3 times a day) on 50 puerperal mothers showed a marked improvement in the flow of milk and fat content when compared to the control group mothers (Anjaria, 1980).

On acute toxicity studies, L. reticulata (aqueous extract) and leptaden administered orally for three alternate days, and three consecutive days to rats, were safely tolerated up to a dose of 3.125 g/kg. An increase in dose led to an increase in mortality. Post mortem, subcutaneous petechial hemorrhage was noted, whilst the liver, kidney and heart showed no apparent change (Anjaria & Gupta, 1967).

**Antifungal activity**

The successive crude chloroform extract of *L. reticulata* root have showed significant antifungal activity against the *Aspergillus niger*, *A. flavus*, *A. terreus* and *Candida albicans*. The maximum zone inhibition was observed in *A. niger* at 300 μg/well. The isolated pure
fraction (lupeol) exhibited significant antifungal activity against the 
*Aspergillus niger*, *A. flavus*, *A. terreus* and *Candida albicans*. The 
maximum zone inhibition was observed in *A. flavus* *A. niger* and *A. 
terreus* at higher concentration of extracts (200 µg/well). While, more 
significant activity *C. albicans* at lower concentration at 100 µg/well 
(Rajanna *et al.*, 2009). Aqueous and ethanolic extracts of *L. reticulata*
roots have been found to have antibacterial action against certain 
strains of *Micrococcus pyogenes* (responsible for certain skin 
infections) and *Streptococcus haemolyticus* (responsible for throat 
infections, rheumatic fever etc), *Salmonella typhi* and *Escherichia coli* 
(Satyavati, 1987).

**Anticancer activity**

Methanol extract of *L. reticulata* roots showed significant anticancer 
activity in anticellogram assay (in vitro: L-929, MIC 25 ppm) (Sukh 
Dev, 2006). *L. reticulata* has been clinically tested and was found 
useful in the treatment of habitual abortion of women (Patel & 

**Anti-implantation activity**

The ethanolic extract of the whole plants of *Leptadenia reticulata* has 
been studied in albino rats to explore it’s the anti-implantation and 
hormonal activities. A strong anti-implantation (inhibition 100%) and 
uterotrophic activity was observed at the dose level of 300 mg/kg and 
*Leptadenia reticulata* posses a significant estrogenic activity shown
by its uterotrop effect in immature female rats and by its ability to increase the weight to genital organs in ovariactomized rats (Rani et al., 2009).

2.3.6 Therapeutic uses

The root of *H. ada-kodien* has various medicinal uses, including as a tonic, an antidiabetic and in opthalmia. The roots of *H. ada-kodien* are reported to possess cooling, alterative, tonic and lactative properties. Made in to a paste, they are applied in ophthalmia and orchitis, they are also used in diabetes, gonorrhea, coughs and stomach-ache (Anonymous, 2001). *H. ada-kodien* root tubers are medicinally important and are useful in opthalmopathy, orchities, cough, burning sensation, stomachalgia, fever and to cure tridosha. The medicinal property is due to the presence of terpenoid sugars and amino acids (Karmarker et al., 2001). *H. ada-kodien* is used for maintaining youthful vigour, strength and vitality (Gupta, 1997).

*Leptadenia reticulata* is much valued in cases of failing eyesight. Both in *Charaka* and *Sushruta Samhitaas L. reticulata* has been classified *jivaniya* (promoter of longevity), *vayahasthaapana* (antiageing) and a *rasaayana* (rejuvenator). The plant is regarded as edible and its preparations are considered good for chest-congestion, cough (*Charaka*), eyesight and for increasing breast milk and secretion of semen (*Sushruta*) (Sukh Dev, 2006). The plant is used in case of mouth ulcers, hepatitis, 'weak heart', tuberculosis, fevers and urinary disorders (Garg, 1965).
L. reticulata is used as a stimulant, galactagogue, oestrogenic, eye tonic, astringent, agalactia and decreased milk after parturition to increase milk, prolapse of uterus, vagina, controlling habitual abortion, maintains pregnancy, induce heat, soothem hard milkers, induce milk letting and useful in skin infections and wounds (Anjaria et al., 1997; Sinha & Sinha, 2001). L. reticulata is useful in the diseases of ear and nose, skin affections, general debility and for increasing milk in cattle. The material is used as ingredient of Sudarshan churna, Chyavanpraasha and some veterinary drug formulations (Sarin, 1999). L. reticulata is stimulant, galactagogue, restorative and tonic. It is used in nasal and ear disorders. The roots are used in skin affections, chest pain (Sharma et al., 2001).

2.3.7 Ethanobotany

An interview study was conducted among natives and tribal people of Gujarat, India by Jadeja et al., (2004) to investigate the ethnobotanical significance of 14 taxa of Asclepiadaceae. The medicinal uses and preparations of the following species are enumerated: Calotropis gigantea, Calotropis procera, Ceropegia bulbosa, Holostemma annularium [H. ada-kodien], Leptadenia pyrotechnica, Leptadenia reticulata, Oxystelma secamone, Pentatropis capensis, Pentatropis spiralis, Pergularia daemia, Sarcostemma acidum, Tylophora dalzelli, T. indica and Wattakaka volubilis. Seven of these were used as vegetable or salad; 4 are used as fodder; and
the rest is used to treat ailments such as abscesses, fever, rheumatism, sprue, asthma, sores, tooth ache, ear ache, snakebite and others.

2.4 Rasna

Things can only be guessed in the case of the identification of Rasna. This Ayurvedic drug is highly controversial and even today proves to be a hard nut to crack. Its Sanskrit synonyms nakuli (liked by mongoose), gandhanakuli (smelled by mongoose), surasa (good to taste), sugandha (aromatic), elaparni (leaves like Cardamom) proved inadequate to track down the botanical source satisfactorily. Rasna of East is different from that of South and North also. Considering the botanical sources which are used for Rasna, the list seems lengthy comprising of Pluchea lanceolata, Vanda roxburghii, Alpinia galanga, Inula racemosa, to mention the prominent ones. The Vaidyas of South India consider Alpinia galanga as Rasna as it is aromatic and the leaves resemble ela. The expert Singh & Chunekar, (1972) has suggested Pluchea lanceolata for Rasna. According to him the synonyms of Rasna may not be unsuitable for the plant considering its external features. Moreover, it possesses some of the regional name similar to Rasna and is being used in rheumatic conditions. Vaidya, (1982) also suggests that Pluchea lanceolata should be taken as Rasna tentatively till one gets better proof to change this.
2.4.1 Distribution

*Alpinia galanga* is native to Indonesia, but has now become naturalized in many parts of India in shady areas. It is cultivated in the Sub-Himalayan region, West Bengal and Assam (Anonymous, 2003; Gupta, 2003). *A. calcarata* is often cultivated in gardens in eastern and southern India for its white flowers, variegated with red and yellow in pyramidal panicles (Anonymous, 2003; Anonymous, 2008). *P. lanceolata* found in sandy or saline soils in Punjab, upper Gangetic plain, Rajasthan and Gujarat (Anonymous, 2003; Billeore et al., 2005; Gupta et al., 2006).

2.4.2 Morphology and Taxonomy

*A. galanga* is a rhizomatous herb, 1.8-2.4 m in height, with tuberous aromatic rootstock, occurring throughout India and cultivated for its rhizome. Leaves oblong-lanceolate, acute, glabrous, 30-60 cm long, ligule rounded; flowers greenish white, streaked with red, in dense-flowered, 30 cm long panicles; capsules orange or red, globose. The rhizome is 2.5-10 cm thick and is reddish brown externally, and light orange-brown inside. It has a tough and fibrous fracture, and a spicy pungent taste (Hooker, 1892; Gamble, 1928; Mohanan, 1984; Anonymous, 2003 Antony, 1989; Mohanan & Henry, 1994; Subramanian, 1995; Sasidharan, 1998; Sunil, 2000; Gupta, 2003). *A. calcarata* is a perennial rhizomatous herb; leafy stem about 1-2.5 m high; leaves linear, lanceolate acuminate,
glabrous, up to 40 x 8 cm; flowers white with red lip in terminal panicle about 15 cm; calyx white tubular, lobed at apex; corolla tube as long as calyx, lateral lobes oblong, apically concave, upper one broader, labellum variegated with red and yellow, to 3 x 1 cm, margin fimbricate, apex rounded; staminodes teeth like, filament flattened, anther cell apically diverging. Ovary densely pilose, 3-celled, ovules many on axile placenta, stigma subglobose (Hooker, 1892; Gamble, 1928; Mohanan, 1984; Antony, 1989; Babu, 1990; Sabu, 1991; Sivarajan & Mathew, 1996; Sunil, 2000; Anil Kumar et al., 2005). *P. lanceolata* is an erect undershrub, 30-60 cm high, stem and branches terete, ashy and pubescent. Leaves sessile, very coriaceous, 2.5-5.7 by 0.6-1.6 cm. oblong or lanceolate, obtuse apiculate narrowed at the base, finely ashy, pubescent on both sides, entire or toothed round at the apex, main nerves prominent. Inflorescence is heads in compound corymbs. Involucres contracted at the mouth. Outer bracts 2-3 seriate; oblong, very obtuse, pubescent, usually tinged with purple; the innermost bracts linear, sub-acute, few pappus hairs distinctly connate at the base (Raghunathan & Mitra, 1982, Tiagi & Aery, 2007).

### 2.4.3 Pharmacognosy

Pharmacognosy of *A. galanga* was reported by Anonymous, (2008), Anonymous, (2006) and Gupta, (2003). In *A. calcarata* this type of work was reported in Anonymous, (2008). Raghunathan & Mitra,

**2.4.4 Phytochemical studies**

*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 principles, the phenyl propanoids 1'-acetoxy chavicol acetate and 1'-acetoxy eugenol acetate from the rhizomes of *A. galanga*. About twelve compounds were characterized in the oil obtained from the rhizomes and leaves of *A. galanga* (Charles et al., 1992). The major compound was myrcene which accounted for 94.51% of the rhizome oil. 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 from the chloroform extract of rhizomes of *A. galanga*, p-hydroxy cinnamaldehyde and (di-(p-hydroxy-cis styril) methane.

Chemical examination of *A. galanga* from Malaysia showed the presence of 1,8 cineole, a 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
along with methyl eugenol, eugenol acetate, chavicol and chavicol acetate (De poorter et al., 1985).

Charles et al., (1992) characterised twelve compounds by GC/MS in A. galanga. The major compound was myrcene, 94.5 1% in rhizome. Barik et al., (1987) reported the pungent principal compound 1'S-l'-acetoxychavicol acetate in Alpinia galanga. The dried rhizomes of A. galanga were extracted with 80% aqueous acetone three times under room temperature. The aqueous acetone extract was subjected to ordinary-phase silica-gel and reversed-phase silica-gel column chromatographies and finally HPLC to give 1'S-l'-acetoxychavicol acetate 1'S-l'-acetoxyeugenol acetate (Noro et al., 1988), 1'S- l'-hydroxychavicol acetate (Lee & Ando, 2001), methyleugenol, chavicol β-D-glucopyranoside (Coen et al., 1995), trans-p-hydroxycinnamaldehyde (Barik et al., 1987), trans-p-hydroxycinnamyl acetate (Loubinoux et al., 1989), trans-p-coumaryl alcohol (Daubresse et al., 1994), trans-p-coumaryl diacetate (Noro et al., 1988).

Akhtar et al., (2002); Someya et al., (2001); Barik et al., (1987); Morita et al., (1988) reported A. galanga contains essential oils, tannins, phlobaphines, glycosides, phenolics and diterpenes. The volatile oil of rhizomes of Alpinia galanga was analyzed by GC and GC/MS. 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 and the composition of
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).

1, 8-cineole (28.4%), α-fenchyl acetate (18.4%), camphor (7.7%), (E)-methyl cinnamate (4.2%) and guaiol (3.3%) are the main constituents of the rhizome essential oil of A. galanga (Jirovertz et al., 2003). Galangoflavonoide isolated from A. galanga rhizome for the first time (Jaju et al., 2009). A. galanga contain rich in essential oil such as cineole, methyl cinnamate, myrecene and methyl euginol and is also said to contain various flavones such as galangin, alpinin, kampferide and 3-dioxy-4-methoxy flavones (Anonymous, 2003; Cui, 2003).

Kaur et al., (2010) reported Hexane, chloroform and ethyl acetate extracts (100 µg/ml) of Alpinia galanga rhizomes exhibited significant activity in vitro against promastigotes of L. donovani. Twelve compounds namely, methyleugenol, p-coumaryl diacetate, I ′-acetoxychavicol acetate, I ′-acetoxyeugenol acetate, trans-p-acetoxyacinnamyl alcohol, trans-3,4- dimethoxyacinnamyl alcohol, p-hydroxybenzaldehyde, p-hydroxyacinnamaldehyde, trans-p-coumaryl alcohol, galangin, trans-p-coumaric acid and galanganol B were isolated from these extracts. Jantan et al., (2004) reported that the rhizome oil of A. galanga from Malaysia is rich in 1,8-cineole (40.5%). Other compounds present in appreciable amounts in oil are the
sesquiterpenoids, β-bisabolene (8.4%), (Z, E)-farnesene (3.8%), β-caryophyllene (3.0%) and (E) β-farnesene (3.2%).

The essential oil contents in various parts of the *A. calcarata* were reported as 0.07-0.10 per cent in the leaves, 0.17-0.25 per cent in the rhizomes, 0.25-0.28 per cent in the root and traces in the stem. Methyl cinnamate was found to be present as a minor constituent unlike in *A. galanga* where this was the major constituent (Rath et al., 1994).

The essential oil of rhizome and leaves of *A. calcarata* revealed the presence of thirty one and twenty eight constituents, respectively. 1,8-Cineole (41.4 per cent and 42.2 per cent) was found to be the major constituent in the leaf and rhizome oil, respectively. Fenchone (0.6 per cent), methyl thymol (0.2 per cent), a-terpinyl acetate (0.4 per cent), valencene (0.2 per cent) and elemol (0.1 per cent) were found in the rhizome oil and not in the leaf oil (Tewari et al., 1999).

Essential oil was obtained from the rhizome of *A. calcarata* in an yield of 0.04 per cent (Choudhury, 1961). The chloroform extract of the rhizome yielded two new phenolic constituents, p-hydroxycinnamaldehyde and di-(p-hydroxy-cis-styryl) methane (Barik et al., 1987). Rath et al., (1994) recorded the physicochemical characteristics of essential oils from the leaves and roots of *A. calcarata*.

In *A. calcarata* rhizome is the potential source of alkaloids/d drugs like alpinine and galagin. The rhizome extract yields 48% methyl
cinnamate, 20-30% cineol, depinine and camphor (Nesamony, 1985). From green rhizomes, pale yellow oil with a pleasant odour can be obtained on distillation. The essential oil yielded 5.6% cineole, 2.6% methyl cinnamate and sesquiterpenes (Joy et al., 2001). Asolkar et al. (1992) isolated 18 monoterpenes of which α-pinene (22.5%), β-pinene (36.7%) and limonene (13.8%) were the major compounds. Kong et al. (2000, 2002) have isolated some diterpenes such as calcaratarins A-E, sesquiterpenes such as shyobunone and coumarins such as hemiarin from the rhizomes of Alpinia calcarata grown in China. Arambewela et al. (2004) isolated 18 volatile constituents in essential oils of Sri Lankan grown Alpinia calcarata rhizomes, roots and leaves. 1,8-Cineol was found to be the major constituent in the oils of rhizomes. Arambewela et al. (2005) reported the essential oils of Alpinia calcarata rhizomes were analyzed for their chemical composition by capillary GC and GC/MS. The major compound in the rhizome 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).

Phytochemical studies on the whole plant of Pluchea lanceolata have led to the isolation of known compounds, β-sitosterol (Hendrickson, 67
1959) and some hydrocarbons, the isolation of known compound propyl 4-hydroxy benzoate (Richards & Hendrickson, 1964). Dasgupta, (1967) has reported the isolation of β-sitosterol, acetyl choline chloride and a quaternary base plucheine from Pluchea lanceolata. Bahl et al., (1968) reported quercetin and isorhamnetin from P. lanceolata.

2.4.5 Pharmacological activities

Expectorant activity

The petroleum ether extract and the volatile oil of the rhizome of A. galanga exhibited expectorant activity in rabbits (Inamdar et al., 1962). The 50 per cent ethanolic extract of the rhizome, in a preliminary biological screening was found to produce gross behavioural effect, antiamphetamine and hypothermic activities in mice. It also showed CVS effect in cat/dog. The extract was devoid of antibacterial, antifungal, antiprotozoal, antiviral, hypoglycaemic and anticancer activities and effect on isolated guinea pig ileum and LD₅₀ of the extract was 1000 mg/kg i. p. in mice (Bhakuni et al., 1969). In another preliminary study, the same sample in addition, showed diuretic activity in rat and was also devoid of antifertility and antiinflammatory activities. The LD₅₀ of the extract was however, 188 mg/kg i. p. in mice (Dhawan et al., 1977).

The drug (A. galanga) has no marked action on other systems of the body. The secretion of urine is slight diminished, but this effect
appears to be vascular, for the rate of secretion comes to normal as soon as the blood pressure comes to normal. The isolated uterus is relaxed and its contractions become regular. The action of the gastro-intestinal tract is similar to that produced by other essential oils (Chopra, 1993).

**Antioxidant activity**

Antioxidant activity of *A. galanga* was measured by DPPH method and β-carotene- linoleic acid method (Vankar et al., 2006). Dose dependent antioxidant activity has been reported in dichloromethane (DCM) and methanol extract of rhizome of *Alpinia galanga* (Vankar et al., 2006). A lot of scientific works have revealed that *A. galanga* and its isolates possess significant antioxidant activity. Essential oil of *A. galanga* has been reported to possess stronger antioxidant activity with IC_{50} value of 550 μg/ml (Tachakittirungrod & Chowwanapoonpohn, 2007). Zaeoung et al., (2005) have reported significant free radical scavenging activity against 1, 1-diphenyl-2-picrylhydrazyl (DPPH) radical in methanolic and water extracts and volatile oils. Antioxidant activity at neutral pH was higher than at acidic pH ranges. Ethanolic extract of galangal has been reported to possess strong superoxide anion scavenging activity. Fe^{2+} chelating activity and reducing power in a concentration dependent manner. However, it also possesses lipoxygenase inhibitor activity (Juntachote and Berahofer, 2005). Dose-dependent antioxidant activity has been reported in dichloromethane (DCM) and methanol extract of rhizome
of *A. galanga* (Vankar et al., 2006). Antioxidative activity reported by Kubota et al., (2001), and xanthine oxidase inhibitory activity (Noro et al., 1988).

**Antitumour activities**

I’S-l’-acetoxychavicol in *Alpinia galanga* possess antitumour activities (Itokawa et al., 1987; Kondo et al., 1993; Moffatt et al., 2000; Zheng et al., 2002) and antifungal activity (Janssen & Scheffer, 1985).

**Anticancer activity**

*A. galanga* exhibited interesting cytotoxic activity. I’S-l’-acetoxychavicol acetate acting as a major cytotoxic component, has shown a significant cytotoxic activity after 48h exposure, against COR L23 cells (lung cancer cell line) and MCF7 cells (breast cancer cell line) with IC₅₀ 7.8µM and 23.9µM, respectively. Due to the relatively high amounts of l’-acetoxychavicol acetate present in the Thai sample. Malaysian galangal showed weak activity as compared to Thai ones (Lee & Houghton, 2005). I’S- l-Acetoxychavicol acetate have been reported to act as an antiulcer and antitumor agents as well as an inhibitor of chemically induced carcinogenesis (Murakami et al., 2000).

**Immunostimulating effect**

A study conducted by Bendjeddou et al., (2003) revealed that a polysaccharide extract of *A. galanga* rhizome possesses a marked stimulating effect on the reticuloendothelial system (RES) and
increased the number of peritoneal exudates cells and spleen cells of mice.

**Inhibitory activity**

Matsuda et al., (2003) reported 80% aqueous acetone extract of the rhizomes of *Alpinia galanga* was found to inhibit release of β-hexosaminidase, as a marker of antigen-IgE-mediated degranulation in RBL-2H3 cells. Antiallergic constituents from natural resources, the aqueous acetone extract of the rhizomes of *Alpinia galanga* was found to show inhibitory activity ($IC_{50} = 19 \mu g/mL$) stronger than those of synthetic antiallergic compounds, tranilast and ketotifen fumarate (Okuda et al., 1984).

**Biphasic activity**

Sadique et al., (1989) studied the bioactivity of certain medicinal plants on the stabilization of RBC membrane system. They concluded that RA (*Rheumatoid arthritis*) formula and its components *Withania somnifera, Pyrethrum indicum, Merendra persica* and *A. galanga* showed biphasic activity in membrane stabilization when fresh sheep erythrocytes were subjected to hypotonic and heat stresses.

**Cytoprotective activity**

Al-Yahya et al., (1990) demonstrated the gastric antisecretory, antiulcer and cytoprotective properties of the ethanolic extract of *A. galanga* in rats. The ethanolic extract at a dose of 500 mg/kg
significantly reduced the intensity of induced gastric mucosal damage in rats. It also significantly reduced gastric secretion and showed marked cytoprotective activity. It is suggested that these properties may be responsible for antiulcer activity of A. galanga. Ahsan et al., (1990) reported the studies of some herbal drugs used against kidney stones. Powdered seeds and rhizomes of A. galanga were tested for their effects on oxalate urolithiasis in male rats. The activity was moderate in A. galanga.

**Toxicity studies**

Qureshi et al., (1992) studied the toxicity of A. galanga rhizome’s ethanolic extracts. Dosages were 0.5, 1.0 or 3 g/kg and 100 mg/kg (daily) in acute and chronic toxicity tests respectively. Chronic administration of the extract increased the body weight. A significant increase in RBC level of treated animals was also observed. Qureshi et al., (1994) examined the effect of A. galanga treatment on cytological and biochemical changes induced by cyclophosphamide in mice. The ethanolic extract of A. galanga rhizomes (125, 250 or 500 mg/ kg) was administered to untreated mice or to mice treated with cyclophosphamide (100 mg/kg).

**Hepatoprotective effect**

A. galanga known to posses gastroprotective activities (Janssen et al., 1985; Jirwan et al., 2006). Hemabarathy et al., (2009) reported the hepatoprotective effect of the crude extract of Alpinia galanga at
200 and 400 mg kg\(^{-1}\) against paracetamol induced hepatotoxicity in rats.

**Anti-inflammatory activity**

*A. galanga* and other plants used under the name ‘rasna’ were tested for comparative antiinflammatory activity against formalin-induced arthritis and carrageenin-induced acute rat paw oedema. The water soluble fraction of the alcoholic extract of the plant was found to be active in chronic arthritis in albino rats. Its antiinflammatory activity was similar to that of betamethasone (Sharma & Sharma, 1977, 1978). In an ethnopharmacological survey for potential antihypertensive from plants, based on an *in vitro* bioassay for angiotensin converting enzyme (ACE) inhibition, it was found that the water, ethanolic and acetone extracts of the rhizome inhibited ACE by 29, 42 and 31 per cent, respectively (Somanadhan *et al.*, 1999).

*p-* coumaryl alcohol-\(\gamma\)-o-methyl ether (CAME) was isolated from *Alpinia galanga* and shown to contain a phenylpropanoid structure similar to *p-* coumaryl diacetate (CAD). CAD is known to have antioxidant and anti-inflammatory activity (Yu *et al.*, 2009).

Pharmacological investigation with the water-soluble fractions of ethanolic extract of the whole plant of *P. lanceolata* was done by (Prasad *et al.*, 1965, 1966) and indicated that, it had two primary actions, acetylcholine-like action and smooth muscle relaxant spasmolytic action, on different muscle preparations. The drug
potentiated the barbiturate hypnosis of the central nervous system. Pluchine isolated by Dasgupta, (1967) from *Pluca lanceolata* have anti-inflammatory action in reducing carragenin induced inflammation in hind paw of albino rats when compared with cortisone taken as standard.


**Hypoglycaemic activity**

Akhtar *et al.*, (2002) reported that administration of powdered rhizome of *A. galanga* to the normal rabbits, at dose levels of 3 and 4/kg produced significant decrease in blood glucose level. However, it could not produce hypoglycaemic effect in alloxan-induced diabetic rabbits.

Aktar *et al.*, (2002) reported in normal rabbits, powdered rhizome and its methanol and aqueous extracts significantly lowered the blood glucose. Gliclazide also produced a significant decrease in blood glucose in the rabbits. In alloxan-diabetic rabbits, *A. galanga* and its methanol and aqueous extracts did not produce significant reduction in blood glucose. The hypoglycaemic effect of *A. galanga* in normal rabbits was comparable to gliclazide. The rhizome was found to contain high levels of certain minerals. Acute toxicity and behavioral studies revealed no visible signs of toxicity and any
abnormal behavior in rabbits even at high doses. It is concluded that *A. galanga* produces fall in blood glucose levels in normal rabbits and the principles, both organic and inorganic, are extractable in methanol and water.

**Antiallergic activity**

*Alpinia galanga* was found to be effective in treatment of allergy (Mastuda *et al.*, 2004). Isolated compounds, 1'S'-1'-acetoxychavicol acetate and 1'S'-1'-acetoxyeugenol acetate from aqueous extract of rhizome have shown to inhibit release of β-hexosaminidase and the antigen-IgE-mediated TNF-alpha and IL-4 production in passive cutaneous anaphylaxis reactions in mice (Matsuda *et al.*, 2003).

**Hypolipidaemic activity**

The ethanolic extract of the rhizomes of *A. galanga* was tested for hypolipidaemic activity in vivo. The extract in a dose of 20 mg/day, administered orally was found to lower the serum and tissue levels of total cholesterol, triglycerides, phospholipids and increased the serum levels of high density lipoproteins in high cholesterol fed white Wistar rats over a period of 4wk (Achuthan & Padikkala, 1995). The rhizome inhibited the lipase enzyme in *in vitro* studies (Vijaya & Vasudevan, 1994).

**Antibacterial activity**

The essential oil obtained from the rhizome of *A. galanga* in a preliminary biological screening revealed antibacterial activity
against *Staphylococcus aureus*, *Escherichia coli*, *Salmonella typhosum*, *Vibrio cholerae* and *Shigella flexneri* (Chopra et al., 1954). The essential oil also showed antibacterial activity against *Shigella dysenteriae* Shigae, *Vibrio cholerae*, *Hemophilus pertusis*, *Salmonella paratyphi*, *Diplococcus pneumoniae*, *Escherichia coli* and *Salmonella schottmuelleri* in an concentration ranging from 0.4 to 0.8 mg/ml. The essential oil had no inhibitory effect on the growth of *Micrococcus pyogenes* var. *aureus* and *Streptococcus pyogenes* even in a concentration of 1 mg/ml.

Thomas *et al.*, (1996) reported antibacterial activity of ether and ethyl acetate extract of *A. galanga*. 1, 8- Cineole, has been reported to have an antibacterial activity against *Staphylococcus aureus* (Gachkar *et al.*, 2007).

The aqueous and the ethanolic extracts of the rhizome of *A. galanga* in a dose of 5-10 mg/ml showed *in vitro* antibacterial activity against *Staphylococcus aureus* and *Streptococcus pyogenes* but was devoid of any activity against *Streptococcus viridans*, *Diplococcus pneumonia* and *Corynebacterium diphtheriae*. The hexane extract of the rhizome was devoid of any antibacterial activity against *Staphylococcus aureus*, *Streptococcus pyogenes*, *Strep viridans*, *Diplococcus pneumoniae*, *Corynebacterium diphtheriae*, *Escherichia coli*, *Salmonella typhi*, *Salmonella paratyphi* A and B, *Shigella flexneri* and *Shigella sonnei* and antifungal activity against *Candida albicans*, *C. tropicalis*, *Piedraia hortae*, *Trichosporon cutaneum*, *Microsporum*...

In a study performed by using broth dilution method ethanol extract of galangal showed the strongest inhibitory effect against *S. aureus* (Oonmetta area et al., 2006). Aqueous extract showed significant activity against *Klebsiella pneumonia*, *Escherichia coli*, *Pseudomonas aeruginosa*, *S. aureus* and *Streptococcus pyogenes* except *Staphylococcus epidermidis* (Turker & Usta, 2002). Essential oil had shown significant activity against *S. aureus*, *Streptococcus suis*, *Erisipelothrix rhusiopathiae*, *P. aeruginosa*, *E. coli*, *Pasteurella multocida* and *Arcanobacterium pyogenes*, with the maximum inhibitory dilution (MID), higher potential in antimicrobial activities was Supposed to be due to the composition 1,8-cineole, 4- allyphenyl acetate and ct.-bisabolene (Tachakittirungrod & Chowwananpoopohn, 2007).

In *Alpinia calcarata* antibacterial activity reported (George & Pandalai, 1949). George & Pandalai, (1949) the antimicrobial screening of *A. calcarata* rhizome EtOH extract and hot water extract was shown antibacterial activity against *Escherichia coli* and *Streptococcus aureus*

**Antiviral activity**

Tewtrakul et al, (2003) reported that methanolic extract of *A. galanga* showed potent inhibitory activity against human immunodeficiency
virus type-1 (HIV-1) and human cytomegalovirus (HCMV).

**Antitubercular activity**

The essential oil from the rhizome of *A. galanga* showed antitubercular activity, completely inhibiting the growth of H<sub>37</sub>Rv (human), H<sub>52</sub>RS (streptomycin resistant), and B<sub>19</sub>·1 (avian) strains, and partially that of B<sub>19</sub>-3 (bovine) strain of *Mycobacterium tuberculosis* in a concentration of 25 μg/mL in in vitro studies.

The essential oil from the rhizome of *A. galanga* was found to be active against *Bacillus cereus*, *Bacillus subtilis*, *Mycobacterium tuberculosis*, *Staphylococcus albus*, *Staph aureus*, *Sarcina lutea*, *Bacillus shigella dysenterae*, *Escherichia coli*, *Shigella boydi*, *Salmonella typhi* and *Vibrio cholerae* (Bhargava & Chauhan, 1968).

**Fungistatic activity**

The essential oil from the rhizome of *A. galanga* showed 100 per cent fungistatic activity against *Trichophyton mentagrophytes* and *Microsporum gypseum* in concentrations of 1000 and 2000 ppm (Dubey & Mishra, 1990).

**Antifungal activity**

In *A. galanga* the alcoholic extract of the rhizome showed *in vitro* anthelmintic activity against human *Ascaris lumbricoides* (Kaleysa, 1975). The hexane extract of the rhizome was devoid of any anthelmintic activity against earthworms *in vitro* (Naqvi *et al*., 1991). Janssen *et al*., (1985) reported the antifungal property of *A. galanga*
due to a compound identified as acetoxy chavicol acetate from the fresh and dried rhizomes. Tripathi et al., (1983) demonstrated the fungitoxic properties of A. galanga oil isolated from the rhizomes of the plant. The oil showed highest toxicity to mycelial growth of Helminthosporium oryzae. The active ingredient of the volatile oil was as fungitoxic as quintozene and zineb and inhibited fungal growth more than did dinocap and copper oxychloride.

Janssen et al., (1985) analysed the different components of the essential oil of A. galanga. When the six main oil components were tested individually, terpinen-4-ol was found to be the most active. An n-pentane + diethyl ether extract of dry rhizomes was active against Trichophyton mentagrophytes. Acetoxy chavicol acetate was active against seven fungi and its minimum inhibitory concentration value for dermatophytes ranged from 50 to 250 μg/ml.

The crude extract of A. galanga showed a good fungal inhibition (60%) of T. longifusus, while a moderate inhibitory activity against A. flavus, M. canis and F. solani (30%, 50% and 40%, respectively) was also observed. A. galanga has been used in traditional medicine for antifungal purposes in Thailand. The A. galanga extract also exhibited 100% inhibition against Lemna minor at highest tested concentration (1000 g/ml) (Khattak et al., 2005). Alpinia galanga shows antifungal activity against Candida albicans (Cheah & Gan, 2000).
A. galanga have shown pronounced inhibitory activities against a wide variety of human pathogenic fungi, including strains resistant to the common antifungal products like amphotericin B and ketoconazole (Ficker et al., 2003). Trakranrungsie et al., (2008) have reported concentration-dependent inhibition of the growth of zoonotic dermatophytes and the yeast-like Candida albicans. Isolated endophytic actinomycetes as Streptomyces aureofaciens CMUAc 130 from the roots of A. galanga, showed significant antifungal activity against Candida albicans and phytopathogenic fungi, Colletotrichum musae and Fusarium oxysporum, at a concentration of 10mg/MI (Taechowisan & Lumyong, 2003). 1'-Acetoxychavicol acetate at a concentration of 14 mg/ml has shown significant activity against Trichophyton mentagrophytes, Trichophyton rubrum, Trichophyton concentricum, Rhizopils stolonifer and Aspergillus niger (Janssen & Scheffer, 1985).

A. calcarata rhizome oil or a mixture of lime and ground rhizome of A. calcarata is used against fungal infection of the skin (Pushpangadan & Atal, 1984). Arambewela et al., (2010) reported that A. calcarata rhizomes possess moderate antioxidant property and promising antifungal activity.

**Antiamoebic activity**

Chloroform extract at a concentration of 1000µg/ml has shown good inhibition against Entamoeba histolytica strains HTH-56: MUTM and
HMI: IMSS (Sawangjaroen et al., 2006). However, it has shown highest activity against *Giardia intestinalis* with the minimum inhibitory concentration (MIC) at 125µg/ml with an IC₅₀, 37.73 µg/ml (Sawangjaroen et al., 2005).

### Gastroprotective activity

Antisecreatory and cytoprotective action of *A. galanga* is responsible for its antiulcer activity. Ethanolic extract significantly reduced gastric secretion in pyrolic ligation and hypothermic restraint stressing rats at a dose of 500mg/kg, whereas, highly significant cytoprotective effect has been reported against 80% ethanol, 0.6M HCl, 0.2M NaOH, and 25% NaCl induced cytodestruction (Al-Yahya et al., 1990). 1'S'-1'-Acetoxychavicol acetate and 1'S-1’-acetoxyeugenol acetate, from *A. galanga* have markedly inhibited the ethanol-induced gastric mucosal lesions in rats (Matsuda et al., 2003); former has shown antiulcer activity in Shay rats (Mitsui et al., 1976). Hisashi et al., (2003) reported the effects of 1’S-1’-acetoxychavicol acetate and related phenylproponoids isolated from the rhizomes of *Alpinia galanga* on ethanol induced gastric lesions in rats. 1’S-1’-acetoxychavicol acetate and 1’S-1’-acetoxyeuginol acetate markedly inhibited the ethanol-induced gastric mucosal lesions. 1’S-1’-acetoxychavicol acetate inhibited the lesions induced by 0.6 M HCL (ED₅₀ =0.73 mg/kg) and aspirin (ED₅₀ =0.69 mg/kg) but it did not show a significant effect on indomethacin-induced gastric lesions and acid output in pylorus-ligated rats at doses of 0.5-5.0 mg/kg.
Anti-platelet activity

*A. galanga* acts as a potential source of platelet-activating factor (PAF) antagonists. In rabbit platelets, methanolic extract showed significant inhibitory effects on PAF with IC$_{50}$ value of 5.5 μg/ml (Jantan *et al*., 2005).

Phytotoxic activity

Khattak *et al*., (2005) reported the ethanolic extracts of *Alpinia galanga* exhibited (100%) phytotoxic activity against *Lemna minor*. These extracts were also found to possess good antifungal activities against *Trichophyton longifusus* (65% and 60%, respectively), while in the brine shrimp lethality bioassay were found to be toxic with LD$_{50}$ of 33 and 109 μg/ml, respectively. Matsuda *et al*., (2003) reported that phenylpropanoids isolated from the rhizomes of *A. galanga* inhibited allergic cytokine IL-4 production and markedly suppressed ethanol-induced gastric lesions in murine models.

*A. galanga* have shown pronounced inhibitory activities against a wide variety of human pathogenic fungi, including strains resistant to the common antifungal products like amphotericin B and ketoconazole (Ficker *et al*., 2003)

Trakranrungsie *et al*., (2008) have reported concentration-dependent inhibition of the growth of zoonotic dermatophytes and the yeast-like *Candida albicans*.

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inhibition against Entamoeba histolytica strains HTH-56: MUTM and HMI: IMSS (Sawangjaroen et al., 2006). However it has shown highest activity against Giardia intestinalis with the minimum inhibitory concentration (MIC) at 125µl/ml with an IC₅₀ 37.73 µl/ml (Sawangjaroen et al., 2005).

Bendjeddou et al., (2003) reported that a polysaccharide extract of A. galanga rhizome possess a marked stimulating effect on the reticuloendothelial system (RES) and increased the number of peritoneal exudates cells and spleen cells of mice.

The petroleum ether extract of A. galanga rhizome in 5 per cent concentration knocked down 88 per cent of housefly Musca nebulo in 10mm and was 100 per cent lethal in 24h (Dixit & Perti, 1963). The LD₅₀ of the oil was found to be 0.068 ml/100 g bw in guinea pigs. In a chronic toxicity study, the oil when given in sublethal doses (0.02 ml/100g) for six weeks did not produce any obvious toxic symptoms in guinea pigs (Chopra et al., 1957).

**Insecticidal activity**

In another study, the essential oil was found to be insecticidal against housefly, Musa domestica nebulo (Abrol & Chopra, 1963). A. galanga rhizome yields an essential oil, called Galangal oil which possesses marked insecticidal properties (Hussain et al., 2006).

**Osteoarthritic activity**

The efficacy and safety of a standardized and highly concentrated extract of Alpinia galanga in patients with osteoarthritis of the knee
were studied. The purified and standardized extract had a statistically significant effect on reducing symptoms of osteoarthritis of the knee (Altman et al., 2001).

**Antinociceptive activity**

Arambewela *et al.*, (2004) reported antinociceptive activities of rhizomes of *Alpinia calcarata*.

**Anthelmintic activity**

*Alpinia calcarata* anthelmintic activity reported (Kaleysa, 1975) reported. The ethanolic extract of the *A. calcarata rhizome* exhibited in vitro anthelmintic activity against human *Ascaris lumbricoides* (Kaleysa, 1975).

### 2.4.6 Therapeutic uses

*A. galanga* is aromatic, pungent and bitter in taste and is used against number of ailments including rheumatism, stomach and bronchial disorders and as a tonic (Perry *et al.*, 1980). The dried rhizome of *A. galanga* provides the drug greater galangal. The drug is used in rheumatism and bronchial catarrh. It is considered a tonic, stomachic, carminative, stimulant, aphrodisiac, used in dyspepsia, fevers, cough and digestive mixtures. The seeds are considered calefacient, stomachic and sternutatory (Nadkarni, 1954; Chopra *et al.*, 1956; Anonymous, 2003).

*A. galanga* is fairly largely used in Southern India. In Mysore, it is a domestic medicine much used by old people with bronchial catarrh.
The rhizomes are useful in rheumatism and catarrhal affections. Tubers and seeds are used as a fragrant adjunct to complex prescriptions. Hakims consider these to be a good remedy for impotence and nervous debility. The drug is a popular remedy for many respiratory ailments. The administration of a paste of *A. galanga* in honey lessened the paroxysms of cough in children suffering from whooping cough and found that in young children suffering from bronchitis, administration of this drug relieved the distressing symptoms and also had a favourable action on the temperature of the patients. The drug, therefore, promises to be of use in respiratory troubles especially those of children. The antispasmodic action of the drug may also prove useful in conditions like asthma. Used also in dyspepsia, fevers, incontinence of urine and also advocated in diabetes mellitus and said to diminish the quantity of urine; it is used to destroy bad smell in the mouth and in other parts of the body; used to improve the voice in throat affections (Nadkarni, 1954). Chopra et al., (1954) reported in affections of the gastio-intestinal tract the drug can be used like other volatile oils. It has got the advantage of having a very pleasant odour and thus may be used in cough and digestive mixtures. It has been-suggested that it may be useful in intestinal and biliary colic. *A. galanga* is used in bronchial catarrh, rheumatism, palpitation of heart and respiratory diseases, especially in the case of children (Sarin, 1999).
*A. galanga* is also used in Arq Pan as a cardiac stimulant and carminative (Takur *et al.*, 1989). The drug stimulates digestion, purifies blood and improves voice Chunekar, (*Bhavaprakasa nigantu*, 1982) *A. galanga* rhizomes are bitter, acrid, thermogenic, aromatic, nervine tonic, stimulant, revulsive, carminative, stomachic, disinfectant, aphrodisiac, expectorant, broncho-dilator, antifungal, febrifuge, antiinflammatory and tonic (Warrier *et al.*, 2002). Rhizome is diuretic and hypothermic (Husain *et al.*, 1992).

Matsuda *et al.*, (2003) rhizomes of *Alpinia galanga* are used as stomachic in China. *Alpinia galanga* is used in Asian (Unani) traditional medicine for treating various diseases included diabetes mellitus (Ikram *et al.*, 1978). *A. galanga* is an essential spice and food flavoring product as well as a medicament or part of medicaments in Asian folk medicine for various applications, such as against rheumatic oilments, for the treatment of respiratory diseases, as aromaticum and tonicum, but also as aphrodisiacum (Purseglove, 1981; De Pooter *et al.*, 1985; Janssen *et al.*, 1985; Charles *et al.*, 1992; Kubota *et al.*, 1998; Kubota *et al.*, 1999; Mallavarapu *et al.*, 2002; Raina *et al.*, 2002).

*A. calcarata* has the similar therapeutic properties of *A. galanga* (Anonymous 1986; Watt, 1989; Kirtikar & Basu 1999).

However, in Indian traditional Ayurveda medicine *Alpinia calcarata* is not generally used. On the other hand, in Sri Lankan traditional Ayurveda medicine rhizome of *Alpinia calcarata* is recommended as
an aphrodisiac and a decoction is widely used in the treatment of bronchitis, cough, respiratory ailments, diabetics, asthma (Ramanayake, 1994) and arthritis (Ramanayake, 1994; Arambewela et al., 2005). Generally drugs that are used for arthrities have antinociceptive and anti-inflammatory properties.

*A. calcarata* are used in the treatment of rheumatism, bronchial catarrh and asthma. It is also used to stimulate digestion, purify the blood, prevent bad breath, and improve the voice and also to treat inflammation (Sharma *et al.*, 1980; Jayaweera *et al.*, 1982). In Sri Lanka *A. calcarata* is commonly prescribed by Ayurvedic physicians along with other plant materials in the treatment of arthritis (Anonymous, 1994; Basnayake *et al.*, 1995).

*P. lanceolata* is employed in drug formulations given in rheumatism and nervous and neuralgic disorders, especially in sciatica. The major preparations are *Raasnaa saptak kwaatha, Mahaamaah taila, Raasnaa panchak kwaatha, Kukuvaadi churna, Maha yograjgugglu* and *Sammirpananga* (Sarin, 1999). *P. lanceolata* is used in indigenous system of medicine in diseases of the rheumatism, allied disorders, and diseases of abdomen, dyspepsia, bronchitis and inflammations (Misra, 1954; and Singh, 1955; Chopra *et al.*, 1956; Cooke, 1958; Kirtikar & Basu, 2006). According to Kirtikar & Basu (2006) the extract is used as an expectorant for bronchitis and asthma and also for the stimulation of digestion, purification of blood and improvement of voice.
*P. lanceolata* is laxative, an analgesic, an antipyretic and a nerve tonic and for the treatment of rheumatism, dyspepsia and bronchitis (Dwived, 1949). The roots are bitter, thermogenic, alexiteric, antipyretic, laxative and used for allaying the pain caused by the sting of scorpions. Externally it is used in rheumatism and also in diseases of the nervous system. The plant is used for the inflammations and bronchitis, cough, psoriasis, piles. It is also used as alaxative, analgesic, antipyretic, nervine tonic. The decoction of plant is used to prevent the swelling of joints in arthritis, inflammations, rheumatism, bronchitis, cough, psoriasis, piles and neurological diseases (Billore *et al.*, 2005; Anonymous, 1969).

### 2.4.7 Ethnobotanical Studies

The rhizome of *A. galanga* is used for skin diseases (Rao & Haridasan, 1991); in rheumatism and in fever. It is also used for stomachache, as a stimulant, carminative and as an aphrodisiac (Ahluwalia, 1968). Two plants ‘kulinjana’ and ‘dveepantara vacha’ mentioned in the Ayurvedic texts, claimed to have various botanical identities according to different authors, have been identified as *A. galanga* according to pharmacolinguistics method of identification (Krishnamurthy, 1971). *A. galanga* is one of the constituents of an Ayurvedic preparation namely ‘rasnadi guggulu’ claimed to be effective in the management of patients suffering from rheumatoid arthritis (Shukla *et al.*, 1985).
The pharmacological details about *A. calcarata* and *P. lanceolata* is very less compared to *A. galanga*. But a lot of therapeutic properties have been mentioned against *P. lanceolata*. A thorough comparative study is required in to come to a conclusion against the controversies.

B. The statement of objectives of the present Investigation:

The concept followed by the present author is given below and the problem can be defined as:

1. Raw Drug 'X' → Source plant A, B, ..., E,

The problem involves equating 'X' with A, B, C, D,or E. This cannot be done arbitrarily. Are A, B, C or E have comparable chemical or pharmacological properties? Whether A......... to E have properties attributed to the raw drug X ?. Whether all these species (A.......E) can be used as the source for 'X'? If not which one(s) ?

2. If 'A' is the genuine source for 'X', and if it is rare or endangered, then what is to be used next? What are the comparable substitutes for A to give quality that is comparable? If the substitute also is rare, is there any substitute’s substitute?

3. What are the characteristics of the source plants of 'X' – botanical, histological chemical and pharmacological characters of A, B or E. This calls for further characterization of A, B,...or E, based on modern techniques to compare the same with 'X'.

The major objectives selected to attempt the problems are:
1. Collection of genuine drugs and their source plants from markets as well as from the natural habitats from various agro climatic regions for pharmacognostic and phytochemical studies.

2. Taxonomic and Micromorphological studies of the raw drugs and their source plants.

3. Preparation of standards of samples based on histochemical technique to find out the genuine source plant from the spurious one, which can be used as a tool to determine the authenticity of raw drugs.

4. Phytochemical profiling of the raw drugs and their source plants based on TLC, HPLC, and GC profiles of terpenoids, steroids, flavanoids and alkaloids.

5. Developing a composite botanical, anatomical and phytochemical profile of the selected plants that can be used as a marker key for quality checking of raw drugs used in ayurveda.

The entire work presented here by the author is the result of her attempt based on the objectives to answer the problem.