Chapter 2  
Review of Literature

2.1 Cancer

Cancer, medically termed as malignant neoplasm, is a class of serious diseases that the proliferating mammalian cells present unlimited abnormal growth behaviors, invasion to adjacent tissues by intrusion and destruction of surrounding matrix, migration from the original location to the other near end sites and finally metastasis through blood and lymph node vessels to distant organs.

Cancer is normally caused by the genetic mutation in transformed cells. It has been evidenced that tumor genesis in the human body is a multistep process, and these reflect inherited alterations, which drive the progressive transformation of normal human cells into highly malignant derivatives.

2.1.1 Epidemiology of cancer

Cancer causes about the quarter of all deaths in developed countries, only slightly less than CVD. The incidence started to increase early in the 20th century. Part of the increase is also undoubtedly due to environmental features of industrialized life such as atmospheric pollution, smoking and diet. There are wide variations in incidence and prevalence of cancer from different anatomical sites, sex, ages, racial, ethnic and geographical groups. These gave tantalizing but largely unresolved glimpses into the etiology of different tumors (American cancer society 2015).

2.1.2 Cell cycle

Normal cells grow and divide in an orderly fashion, in accordance with the cell cycle. The cell cycle is an ordered process of events that occurs in four stages. During the two gap phases, G1 and G2, the cell is actively metabolizing but not dividing. In S (synthesis) phase, the chromosomes duplicate as a result of DNA replication. During the M (mitosis) phase, the chromosomes separate in the nucleus and the division of the cytoplasm (cytokinesis) occurs. There are checkpoints in the cycle at the end of G1 and
G2 that can prevent the cell from entering the S or M phases of the cycle. Cells that are not in the process of dividing are in the G0 stage, which includes most adult cells (Pucci et al.2000).

Cell cycle is regulated by two families of molecules known as cyclins and cyclin dependent kinases (CDKs). Cyclins bind to CDKs and regulate target protein required for entry into the next phase of a cell cycle. Multiple check points monitor and regulate the progress of the cell cycle. These check points prevent the cell cycle from progressing, if certain requirements have not been met, allowing verification of the necessary phase process and repair of defective DNA products. Two main check points G1/S and G2/M check points. Cell cycle is also regulated by genes, which prevent its progression such as p53, p21 and p16. These play an important part in tumor prevention and are known as tumour suppressor gene. They halt the cell cycle by binding to and deactivating cyclic – CDK complexes. Disregulation of any part of the cell cycle may be associated with increased susceptibility to cancer.

2.1.3 Genetics of cancer

Cell growth of normal as well as abnormal types is under genetic control. In cancer, there is either abnormality in genes of the cell or there are normal genes with abnormal expression. The abnormality in genetic composition may be from an inherited or induced mutation (chemical, viruses or radiation). The mutated cells transmit their characters to the next progeny of cells and results in cancer. In normal cell growth, regulatory gene's control mitosis, as well as aging and cell death by apoptosis.

In normal cell growth, there are four regulatory genes,

- Proto-oncogenes are growth promoting genes.
- Anti-oncogenes are growth inhibiting or growth suppressor genes.
- Apoptosis regulatory genes control programmed cell death.
- DNA repair genes regulate the repair of DNA damage that has occurred during mitosis and also control the damage to proto-oncogenes and anti-oncogenes.
In cancer cell, the abnormalities in controlling genes may occur due to

- **Activation of growth promoting oncogenes**

The conversion of a proto-oncogene to an oncogene may occur by mutation of the proto-oncogene, by rearrangement of genes in the chromosome that moves the proto-oncogene to a new location, or by an increase in the number of copies of the normal proto-oncogene. Sometimes virus inserts its DNA in or near the proto-oncogene, causing it to become an oncogene. The result of any of these events is an altered form of the gene, which contributes to cancer.

- **Inactivation of cancer suppressor genes**

Inactivation of anti-oncogenes removes the growth control of cells and is believed to be a key factor in the development of several tumours (retinoblastoma, breast cancer, lung carcinoma, Wilm’s kidney tumour). Anti-oncogenes are active in the recessive form which means that they are active only if both alleles are damaged.

- **Abnormal apoptosis regulatory genes**

Genetic damage of genes that regulate programmed cell death also contributes to the development of cancer. Bcl-2 a proapoptotic gene, involved in the apoptosis and angiogenesis.

- **Failure of DNA repair genes and thus inability to repair the DNA damage resulting in mutation.**

Environmental factors, such as ionizing radiation, UV light, and chemicals, can damage DNA. Errors in DNA replication can also lead to mutations. Certain gene products repair damage to chromosomes, thereby minimizing mutations in the cell. When a DNA repair gene is mutated its product is no longer made, preventing DNA repair and allowing further mutations to accumulate in the cell. These mutations can increase the frequency of cancerous changes in a cell.
Table 2.1 Common oncogenes altered in human cancer.

<table>
<thead>
<tr>
<th>Oncogene</th>
<th>Neoplasm</th>
<th>Oncogene</th>
<th>Neoplasm</th>
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<tbody>
<tr>
<td>Bcl2</td>
<td>B cell lymphoma</td>
<td>RET</td>
<td>Thyroid cancer</td>
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<tr>
<td>PDGF</td>
<td>Brain tumour</td>
<td>BC11</td>
<td>Head and neck cancer</td>
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<tr>
<td>N-Ras</td>
<td>Leukaemia</td>
<td>K-Ras</td>
<td>Ovarian and lung cancer</td>
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<td>c-Myc</td>
<td>Leukaemia</td>
<td>L-Myc</td>
<td>Lung cancer</td>
</tr>
<tr>
<td>N-Myc</td>
<td>Glioblastoma</td>
<td>APC</td>
<td>Stomach and colon</td>
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<tr>
<td>WNT1</td>
<td>Retinoblastoma</td>
<td>HRAS</td>
<td>Colon, Lung, Pancreas</td>
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<tr>
<td>KRAS</td>
<td>Melanoma, colorectal cancer</td>
<td>REL</td>
<td>Lymphomas</td>
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<tr>
<td>JUN</td>
<td>Lung Cancer</td>
<td>WTI</td>
<td>Wilm's tumour</td>
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<tr>
<td>BRCA1</td>
<td>Breast and ovarian</td>
<td>BRCA2</td>
<td>Breast cancer</td>
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<tr>
<td>VHL</td>
<td>Renal cell cancer</td>
<td>FOS</td>
<td>Osteosarcoma</td>
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(Fauci et al 2008)

2.1.4 Metastasis of cancer

Tumour metastasis is are responsible for approximately 90% of all cancer related death. This involves molecular networks that promote local cancer-cell invasion, single-cell invasion, formation of the metastatic microenvironment of primary tumours, intravasation, lymphogenic metastasis, extravasation, and metastatic outgrowth. Altogether, these functional networks of molecules contribute to the development of a selective environment that promotes the seeding and malignant progression of tumourigenic cells in distant organs. When cancer is detected at an early stage, prior to its wide spreading, surgery excision or irradiation with the primary tumour, followed by chemo- or immune- therapies could have successful treatment effects to patients. However, when cancer is detected to be in metastatic stages, such treatments are much less effective. Furthermore, those patients in whom there is no evidence of metastasis at the time of their initial diagnosis, metastases could still be detected in a later time along with the cancer recurrence. The breast cancer, for example, can reappear with metastatic growth even years after the patient has been declared cancer free (Weber et al. 2005). At the time of initial diagnosis close to a one-third of breast cancer patients are positive for metastatic disease (Jemal et al. 2007). This clearly shows that a subpopulation of tumour cells developed therapy resistance, spread, survived at secondary distant sites to recommence uncontrolled growth. The transformation is an evolutionary process from a
non-malignant to a malignant metastatic phenotype, where tumour cells acquire aggressive characteristics progressively that results from, or a consequence of, selection of a sub-population of cells that are ultimately capable of completing all the metastatic cascade steps (Wyckoff et al. 2000; Merlo et al. 2006; Butler & Gullino 1975).

Figure 2.1 Metastasis from malignant primary tumour to distant site (Adapted from Nature Reviews Cancer vol.17, 737-749).

After dissemination, tumour cells are believed to be regulated by their immediate microenvironment, whereas there is increasing evidence that pre-conditioning of arresting sites may occur before tumour cells reach the secondary site. It is believed that some of the same steps as in primary tumour growth is followed in the formation of a tumour mass at the secondary site. ‘Colonization’ is the term used herein to implicit the combined influences of tumour cell proliferation, apoptosis, dormancy and angiogenesis in the formation of a progressively growing lesion in a distant organ. However, the major distinction is that these disseminated cells grow in an ectopic environment. Hence, only those cancer cells- that are able to adapt to the fresh environment - or those cells that can modify the new microenvironment can be expected to thrive (Paget 1989).
2.2 Angiogenesis in cancer

Angiogenesis, the formation of new blood vessels from the pre-existing capillaries, is one of the vital events for organ development and differentiation during embryogenesis as well as wound healing and reproductive functions in the adult (Folkman 1995). During embryonic development, new endothelial cells differentiate from stem cells, through a process called vasculogenesis. In angiogenesis, new blood vessels primarily emerge from pre-existing ones. In adult life, physiologic stimuli during wound healing and the reproductive cycle in women lead to angiogenesis, whereas vasculogenesis is absent (Battegay 1995). However, angiogenesis also underlines some pathophysiologic conditions, such as cancer, rheumatoid arthritis, diabetes mellitus, and coronary artery disease, etc. (Nyberg et al. 2005). Typically in cancer development, tumour stimulates angiogenesis by directly secreting angiogenetic substances or by activating and releasing angiogenetic compounds stored in the extracellular matrix (Hanahan & Folkman 1996). Tumour associated vasculogenesis can also be enhanced by angiogenetic substances secreted by tumour lymphocytes, macrophages, and/or mast cells (Cavallo et al. 2011). This leads to the activation of nearby endothelial cells, which respond by expressing a cell autonomous pattern of behavior that culminates in the formation of new vessels. Figure 2.2 shows the individually divided steps in the angiogenesis process.

![Figure 2.2](image)

**Figure 2.2** Angiogenesis steps in tumour microenvironment. Release of angiogenetic substances by tumour cells results in the tumour endothelial cell activation and formation of new blood vessels (Adapted from The Journal of Urology, vol. 158, 1663-1674).
Once activated, endothelial cells first lead to proteolytic disintegration of basement membrane in order to create a blood supply to growing tissues, through a process that is facilitated by proteases. Next, chemo-attractants and mitogens are activated in order to induce migration of cells into the extracellular matrix and their proliferation. Finally, vessel formation is completed by differentiation and/or apoptosis of endothelial cells, recruitment of accessory cells, and deposition of extracellular matrix (Hanahan 1997; Conway 2001). Then, endothelial cells acquire organ specific properties, such as cell-surface markers and/or morphological characteristics according to their environment and interactions with adjacent cells (Auerbach et al. 1976). Each step is essential to the formation of tumour associated neovascularature and represents a potential site of therapeutic manipulation.

2.2.1 Angiogenesis, tumour growth and metastasis

In the past three decades, it has been evidenced that solid tumours must induce angiogenesis to grow and metastasize. Tumours smaller than 2 to 3 mm in diameter can obtain adequate nutrient support by diffusion from the existing vasculature, but further expansion requires the induction and growth of new capillaries (Folkman 1992). Experimental tumours, for example, can hardly grow when implanted into nonvascular areas, such as the interior chamber of the eye. Nevertheless, when placed against the iris, where angiogenesis can occur, they can grow aggressively to cover the entire eye (Gimbrone et al. 1972). Tumour growth rate is decreased dramatically when the mice are treated with agents who specifically inhibit angiogenesis, even though these agents have no direct effect on the tumour cells themselves (Kim et al. 1993). In contrast, tumour growth could be stimulated by treatment with pro-angiogenesis factors.

Angiogenesis is also critical in tumour metastasis. Tumour cells can hardly extravasate into the circulation system significantly until angiogenesis has been induced. In addition, metastatic cells seeding at new organs must attract new vessels before they can grow to a clinically significant size.
2.2.2 Angiogenesis inhibitors

As early as 1971, Folkman first hypothesized that tumour growth is angiogenesis dependent, and the inhibition of the tumour neo-vasculature could suppress tumour growth and/or metastasis (Folkman 1971). Nowadays, numerous strategies for modulation of angiogenesis are reported. Angiogenesis mainly depends on proper activation, proliferation, adhesion, migration, and maturation of tumour endothelial cells. Most approaches to modulate angiogenesis are therefore focused on these endothelial functions during the angiogenesis process.

**Intervention of tumour endothelial cell growth:** One of the successful methods for angiogenesis inhibition lies in the use of agents who specifically inhibit the tumour endothelial growth. One of the first compounds identified to show inhibitory effects on endothelial cell growth is TNP-470, an analog of the fungus-derived antibiotic fumagillin (Ingber et al. 1990). The action mechanism of this compound was found to prevent endothelial cells from entering G1 phase of the cell cycle, resulting in a decrease in proliferation (Locigno et al. 2000). In a later time, many endogenic molecules with angiostatic activity were discovered, such as thrombospondin-1, interferon-inducible protein-10 (Luster 1995), etc. Among the endogenic anti-angiogenesis inhibitors, both endostatin and angiostatin have been widely studied and different phases of clinical trials are undergoing. It has high affinity for all isoforms of VEGF and causes no immune response in humans (Semaxanib), a specific inhibitor of VEGFR-2 phosphorylation, or SU11248 (Sutent®, Pfizer), a multi receptor tyrosine kinase inhibitor of VEGF receptor cellular signaling (Potapova et al. 2006).

**Intervention with endothelial cell adhesion and migration:** Because interaction between endothelial cell and extracellular matrix is highly involved in the angiogenesis progress for endothelial cell adhesion and migration, many efforts have been paid on this step for anti-angiogenesis purpose. Interferon (IFN), the first identified endogenous angiogenesis inhibitor, regulates the angiogenesis by inhibiting the tumour-induced motility of capillary endothelial cells as well as the spontaneous migration of other cell types (Fong et al. 1999).
Another important molecular family which is involved in angiogenesis progress through endothelial cell interaction with extracellular matrix is the Integrin family. Integrin’s are cellular surface glycoprotein receptors consisting of a heterodimer of α and β subunit, which are mutually non-covalently associated.

2.3 Medicinal plants as anticancer agents

Over the past century, a number of natural compounds extracted from animals, plants, microbes and marine organisms have been used to treat human diseases. The endeavour involved well-known drugs such as penicillin (antibacterial), morphine (analgesic), artemisinin (antimalarial) and paclitaxel (anticancer). Despite increasing competition from combinatorial and classical compound libraries, there has been a steady introduction of NP-derived drugs in the last ten years. A total of 19 NPs-based drugs were approved for marketing worldwide between 2005 and 2010, covering infectious (bacterial, fungal, parasitic and viral), immunological, cardiovascular, neurological, inflammatory and related diseases, and oncology (Chen et al. 2015).

Plants have been a prime source of highly effective conventional drugs for the treatment of many forms of cancer, and while the actual compounds isolated from the plants frequently may not serve as drugs, they provide leads for the development of potential novel agents. As new technologies are developed, some of the agents, which failed in earlier clinical studies, are now stimulating renewed interest.

There are several clinically useful compounds derived from plant products.

Paclitaxel, a mitotic inhibitor was isolated originally from Taxus brevifolia. Cabazitaxel, larotaxel, ortataxel, milataxel, tesetaxel and taxoprexin are currently in use in the treatments of patients with pancreatic, prostate, breast, and hormone refractory prostate cancers.

3’-O-methyl-nordihydroguaiaretic acid (NDGA), a lignan isolated from the Larrea divaricatta is known to exhibits significant antipromoter, anti-inflammatory and antineoplastic activities. Terameprocol, a synthetic derivative of NDGA, induces
apoptosis in cancer cells and are evaluating in various Phases against solid tumours, glioma and leukaemia.

Ingenol extracted from the sap of *Euphorbia peplus* is under clinical development by Peplin Biotech for tropical treatment of certain skin cancers such as basal cell carcinomas and squamous-cell carcinoma. Ingenol mebutate, a PKC activator and derivative of Ingenol are currently in clinical development against acinic keratosis (AK).

Homoharringtonine, a myelosuppressive alkaloid originally isolated from *Cephalotuxus fortuneii* induces apoptosis against acute myeloid leukaemia. An orphan drug designation was given to this drug by FDA to use in the treatment of myelodysplastic syndromes.

Daidzein, an isoflavone occurring in a number of plants and herbs, including *Pueraria mirifica*, soya beans and soya products, exhibits clinical indication against tumours. Phenoxodiol, a synthetic analogue, as a chemosensitizing agent in combination therapy with platinum drugs against chemoresistant ovarian cancer and also in the monotherapy to use in the treatment of prostate and cervical cancers. An orphan drug status was granted to Phenoxodiol, by the FDA for cholangiocarcinoma, advanced prostate and melanoma. Triphendiol is an orally delivered chemosensitizing derivative of Phenoxodiol, is under the phase trials in combination therapy. Genistein, a soya derived antineoplastic phytoestrogen, inhibits protein-tyrosine kinase and induces cell differentiation. It is also supported to inhibit topoisomerase II resulting in DNA fragmentation and apoptosis.

Vadimezan, a derivative of flavone-8-acetic acid is β-Lapachone, a naphthoquinone obtained from *Tabebuia avellanedae* exerts the antitumour effect by a rapid and sustained increase of the pro-apoptotic protein E2F-1 as well as induces expression of cyclin dependent kinase inhibitor.

Epipodophyllotoxin, a naturally occurring lignan extracted from the root of *Podophyllum peltatum*, is a dual inhibitor of both topoisomerases, I and topoisomerases
II. Tafluposide is a derivative of Epipodophyllotoxin and is currently in clinical development for antitumour activity by Pierre Fabre.

Gossypol, a polyphenolic aldehyde extracted from cottonseed plant of genus *Gossypium* acts as an inhibitor of several dehydrogenase enzymes. Ascenta Therapeutics announced the promising results in prostate, brain and lung cancers.

Curcumin, a polyphenol extracted from roots of *Curcuma longa* has been considered significantly against metastatic colon cancer due to its ability to interfere with the p53 tumour suppressor pathways. It is under various trials worldwide.

Betulinic acid, a pentacyclic triterpenoid isolated from *B. pubescens* was evaluated in clinical trials against malignant melanoma (MM) and has orphaned drug designation by the FDA for tropical treatment of MM.

Bardoxolone methyl, a synthetic triterpenoid analogue of oleanolic acid occurring naturally in various food and medicinal plants under clinical development against prostate cancer and phase II dependent with chronic kidney disease (Mishra & Tiwari 2011).

**Lignans as cytotoxic agents:** Lignans are widely distributed in angiosperm and gymnosperm. Various lignans are known to have anti-tumour, antimitotic and anti-viral activity. Lignans have been isolated from all parts of plants; tree bark, wood of angiosperm trees, resins, roots, leaves, flowers, fruits and even seeds. Lignans have aroused considerable interest because some of them display antineoplastic activity. It is difficult to identify a common structural characteristic which might explain their activity as antitumour agents. Many of the compounds share the features like i) a five-member lactone ring, ii) 3, 4, 5-trimethoxy phenyl group, iii) a methylenedioxy group and iv) two substituted phenyl groups separated by a four carbon group. It is interesting to note that methylenedioxy phenyl group is suggesting its importance in eliciting its cytotoxic response (MacRae & Towers 1984).
2.4 *Justicia simplex* D Don. (Syn: *Justicia japonica*)

*Justicia simplex* is an erect, shrub or under herb, up to 50 cm in length, seen at grass lands and waste places. It belongs to the family of *Acanthaceae* (Podromus Florae 1825). It is distributed in Nepal, India, Pakistan, Sri Lanka, Burma and Thailand and is seen at an altitude of 700-2500 m. The leaves of *J. simplex* ovate/oblong or lineate, flowers are sessile or sub sessile in spikes with pale, pink or purplish. The branches have been pale soft hairy herbaceous, diffuse branching often quadrangular, zigzag (Hooker 1885; Gamble JS 1989) Traditionally used as a stomachic, diuretic and anthelmintic, it removes indigestion, toothache and vomiting (Kanakappan & Balakrishnan 2009).

The phytochemical analysis of the *J. simplex* extracts showed the presence of alkaloids, protein, flavonoids, amino acids, tannin, carbohydrates, saponins, terpenoids and steroid in the aqueous extract. The investigation of the extracts of *J. simplex* revealed that ethanol extracts had a promising antibacterial activity against dental bacterial pathogens (Eswari et al. 2014). Jasmine et al., reported that crude extracts of *J. simplex*, and its isolated compounds, simplexolin and sesamolin have protective effect on CCl₄ induced liver damages (Jasmine et al. 2007). Crude ethanol extract of *J. simplex* caused an IC₅₀ value of 200 mg/kg in the Brine shrimp lethality assay, whereas the aqueous extract exhibited greater than 1000 mg/kg (Cantrell et al. 2003). A new triterpenoid saponin, named justicisaponin-I, has been isolated from *J. simplex*, which produced significant sperm acrosomal membrane stabilizing action and may prove to be a potent antifertility agent (Ghosal et al. 1981). A furofurano lignan, justisolin and simplexoside were also isolated from the petroleum ether extract of *J. simplex* (Ghosal et al 1980). By extensive chromatography of petroleum ether extract of *J. simplex*, three known lignans-asarinin, seasamin, sesamolin and a lignan simplexolin were isolated (Ghosal & Banerjee 1979).

2.5 *Myxopyrum smilacifolium* (Wall) Blume

*Myxopyrum smilacifolium* is a small genus, large woody climber, which belongs to the family of Oleaceae, commonly known as, Malayalam - Chaturavalli, Tamil- Chaturamullai, Sankrit- Hemamalathi or Chaturalatha. It is distributed in the forest of
Bangladesh, India, Cambodia. (Sasidharan 2004). It has four angled branches. Leaves are opposite, ovate or elliptical, serrulate, 10-16 cm in length, 4-10 cm diameter. Flowers are small, bisexual and yellow in colour. Ovoid berries with one or two beads (Hooker 1885). Roots of this plant are useful in scabies and purigo in children. The leaves are astringent in taste, used in the vitiated conditions of Kapha and vata. Powdered leaves with ghee are used as a remedy for asthma, rheumatism. Leaves boiled in oil are applied in fever, headache, ear diseases and backache. This plant is used successively as a remedy for the treatment of inflammatory conditions (Sastri 1962; Warrier 1983).

Preliminary phytochemical screening of the leaves of *M. smilacifolium* revealed the presence of alkaloids. HRBC and Protein denaturation assay proved the *in vitro* anti-inflammatory activity of the plant (Samu et al. 2014) and also showed potent *in vivo* anti-inflammatory action. Root extracts revealed the presence of alkaloids, phenolics, glycosides, tannins and flavonoids (Hill et al. 2012). An iridoid glycoside, Myxopyroside was isolated from the leaves of *M. smilacifolium* (Franzyk et al. 2001).

**2.6 Memecylon malabaricum Cogn.**

*Memecylon malabaricum* of family Melastomataceae is a tree up to 5 m tall commonly known as Malayalam- Kasavu, Kayavu; Tamil-Malamthetti, Kannada-Mundi. It is distributed to Western Ghats. It can be seen commonly in evergreen forests up to 2400 m (Sasidharan 2004). The leaves are simple, opposite; flowers are blue in colour; fruits are berry, and seeds are globose seeds. They are used against bacterial infection, inflammation and skin diseases traditionally (Journal of natural history Society 1989).

A study on ten genera of Melastomataceae family revealed that it mainly contained tannins, flavonoids, amino acids, fatty compounds and terpenoids, etc. They have hepatoprotective, hypoglycaemic, hypotensive, Maillard reaction-inhibiting, antioxidation, antimicrobial, antiviral, cytotoxic, contraceptive, smooth muscle contraction-inhibiting, anti-inflammatory and haemostatic activities (Guan 2006).

The petroleum ether, chloroform and methanol extracts of *Memecylon malabaricum* leaves were tested for antimicrobial activity. Only methanol extracts gave activity against bacteria both Gram (+) and Gram (-), and fungi (Hullatti & Rai 2004).
2.7 *Litsea quinqueflora* (Dennst)

These are tropical and subtropical trees /shrubs, usually seen in dry evergreen forests between 700-2200 m (Gamble J S 1989). Leaves are simple, alternative; flowers in auxiliary, fruits are berry and the seeds are glaborous (Hooker 1885). It belongs to the family of Lauraceae having the synonym *Litsea lingustrina*. It is known as Kuttypannel in Malayalam. This plant is endemic to Western Ghats.

Decoctions of stem bark with little sugar are used against dysentery, whooping cough and chickenguniya traditionally. Preliminary phytochemical screening of the leaves of *L. quinqueflora* revealed the presence of flavonoids, coumarins, alkaloids, tannins, terpenoids, anthraquinones, phenols, reducing sugars and carbohydrates. *In vitro* anti-inflammatory activity was evaluated by HRBC membrane stabilization method with slight modifications (Anilkumar & Jibin 2015).
Figure 2.3 Images of selected plants

- *Justicia simplex* D Don.
- *Myxopyrum smilacifolium* (Wall) Blume
- *Memecylon malabaricum* Cogn.
- *Litsea quinqueflora* (Dennst).