CHAPTER 1
INTRODUCTION SCOPE OF THE STUDY, OBJECTIVES AND REVIEW OF LITERATURE

1.1 INTRODUCTION

The current pharma industries are focusing on plants for the preparation of novel medicines and the available 25% medicines are compounds from plants or similar compounds prepared on the basis of plants origin compounds (Atanasov et al. 2015). The third president of USA, Thomas Jefferson explained that, “The greatest service which can be rendered to any country, is to add a new useful plant to its culture”. In the twenty first generation people completely depend on the plants, not only for the food, but for medicines and the plant biotechnology gives new diversion for the synthesis of medicines from plants (Cragg and Newman 2013). India, a country with 15 different agro climatic zones where more than 15,000 medicinal plants have been recorded alone for the 7000 plants having medicinal usage in common folk and documented systems of medicine (Parasuraman et al. 2014). Plants are used in Ayurveda, Siddha, Unani, and Homoeopathy to treat many diseases.
It was reported very earlier that 80% problems of the world inhabitants are treated by medicinal herbal drugs (Singh and Lal 2008). *Averrhoa bilimbi* L. belongs to Oxalidaceae family and traditionally its fruits are used in pickle preparations (Rahman 2003). The leaves, flowers, and fruits are used to treat a cough, pimples, hypertension, diabetes, fever, swellings, inflammation, and to stop rectal bleeding (Zakaria et al. 2007). *Averrhoa bilimbi* L. is wealthy for its source of minerals such as Phosphorous, Nitrogen, Potassium, and Iron (Dangat et al. 2014). It has antimicrobial activity (Das et al. 2011) and antidiabetic property (Pushparaj et al. 2000). In the Philippines, the leaf is being applied as a paste on itches, swelling, Rheumatism, Mumps or skin eruptions and used for bites against poisonous insects (Patel et al. 2012).

*Averrhoa* genus mostly grows in the tropical moist regions, which belongs to oxilidacea family. Different variety of *Averrhoa* species are available in which, only *Averrhoa bilimbi* and *Averrhoa carambola* are the two plants which give fruits (edible to eat) having biological activity (Ken Love1 et al. 2017). The different parts of *Averrhoa bilimbi* L. showed anti-cholesterolemic activity (Ambili et al. 2009); antibacterial and antioxidant activities (Kumar et al. 2013). The fruits of *Averrhoa bilimbi* L. are good source of minerals like Nitrogen, Nitrate, Phosphorous, Potassium, Calcium, Magnesium, Sulphur, Zinc, Ferrous, Copper, Manganese, Molybdenum, Boron (Dangat et al. 2014) and Vitamin C (Ariharan et al. 2012). The enhancement of the (ROS) Reactive oxygen species and (RNS) Reactive Nitrogen Species will damage the cells and elaborate the free radical system by dominating the antioxidant defence mechanism in the body. The minerals available in the fruits act as scavengers for these (ROS and RNS). The minerals like Sodium, Potassium, Calcium, Magnesium and Phosphorous play a major role in the muscular and cardiac contraction, apoptosis and oxidative stress (Houston 2010). For the athletes Zinc, Magnesium and Calcium helps in the immune
function (Speich et al. 2001). Vitamin C has antioxidant property which scavenges the free radicals (Sebastian et al. 2003). The regular intake of Vitamin C was reported to reduce the risk of cancer particularly colorectal cancer that can be controlled by Vitamin C (Car and Frei 1999).

Based on the little available information, we tried to find out the protective and curative properties of the shade dried *Averrhoa bilimbi* L. fruit extract in the experimentally induced *invivo* condition of Ulcerative Colitis and Ascitic Lymphoma. Furthermore its antioxidant property and *in vitro* cytotoxic activity on human colon cancer cell line were analysed in this study.

Ulcerative Colitis (UC) is an inflammatory stipulation where large intestine and the inner colon lining regions are affected with chronic inflammation. The patients with UC have the ulcers (sores) and inflammation at inner linings of colon, which leads to diarrhea and rectal bleeding. Inflammatory Bowel Disease (IBD) is a combination of the Crohn’s diseases (CD) and Ulcerative Colitis, where CD affects from mouth to anus region where the gastrointestinal tract is present and UC affects the inner linings of the colon and rectum or mucosa leading to the inflammation and ulceration (Baumgart and Carding 2007). The initiation and activation of the UC is still unclear. Free radical accumulation and oxidative stress in the body play a significant role in the initiation and progression of UC (Kruidenier and Verspaget 2002).

The abnormal condition of the immune system in the colon region, environmental factors, genes and bacteria (*Helicobacter pylori*) are the major reasons for the UC (McGuckin et al. 2009). The abnormal changes in the levels of inflammatory immune cells and factors such as cytokines and interleukins
are the hallmarks of UC (Hiraki et al. 2004). The severity of the UC leads to the colon cancer. The risk of colon cancer development is high, in the patient who is affected frequently by the UC (Levin et al. 1991).

The abnormal uncontrolled division of cells and escaping from the process of apoptosis (Cell death) is called as cancer. The abnormal cells do not respond to the signals of cell death or control on cell division and spreads to other parts of the body (metastasis) (Baylin and Jones 2011). Even though the advanced treatments are able to cure initial stages of cancer, the metastasis is the most devastating and dangerous aspect of cancer, demanding the most challenging therapies. The medication for metastasis is still unknown and is the major cause of cancer deaths. One of the hallmarks of metastasis is the migratory ability of cancer cells. Hence we analysed the effect of *Averrhoa bilimbi* L. fruit extract on inducing apoptosis and inhibiting migration of human colon cancer cells *in vitro* in this study.

At the site of cancer tumor development, the inflammatory tumor-infiltrating cells produce wide varieties of cytokines (TNF-α, COX-2) and interleukins (IL-6, IL-1β). The alterations in the cytokines and interleukins contributes to carcinogenesis and metastasis (Hiraku et al. 2004) i.e. the mutation rates in the body enhances through the inflammatory microenvironment which escalates the procreation of the mutated cells. The reactive oxygen species (ROS) and reactive nitrogen intermediates (RNI) are proficient in evoking the damage to DNA along with genomic instability which are the major outcomes of the activated inflammatory cells (Grivennikov et al. 2010). Superior level of the reactive oxygen species (ROS) and Reactive Nitrogen species (RNS) are one of the major causes for cancer. This ROS and RNS have the free electron in their outer orbit and try to react with the other
normal proteins and DNA in the body so far to alter the normal function of the body (Liou and Storz 2010). Thus we understand from the literature review that there is a good correlation between the oxidative stress mediated reactive species, inflammatory mediators of UC and colon cancer.

The available medication for the UC is corticosteroids, antibiotics and antibodies for tumor necrosis factors (TNFs). These treatments are having side effects like hypertension, nausea, hypertrichosis, headache and renal insufficiency (Meier and Sturm 2011). Similarly chemotherapy, surgery and radiation therapy are the notable treatments available as cancer therapy. All these treatments are also connected to adverse side effects leading to a decrease in the quality of human life. It’s time to develop a new alternate source of treatment to cure disease without any adverse side effects. From time immemorial, plants are the best source for medication to treat multiple diseases. The compounds from the natural source (Plants), named phytochemicals are helpful for the well-being of human life and serve as therapeutic drugs or as phytopharmaceuticals. Several of the available phytochemicals from different plants such as curcumin as anti-ulcerative (Hanai et al. 2006), quercetin as anti-cancer (Yu.Jue et al. 2015) are being used for such patients. Epicatechin gallate and Ellagitannins of polyphenols showed anticarcinogenic properties (The Epicatechin gallate induced apoptosis in colon SW480 cells was associated to down-regulation of the PI3K/AKT pathway and the stimulation of MAPKs signalling and p53. The activation of the JNK and p38 increased the rate of p53 levels and lead to the p53 amplification). It was suggested that such studies on mechanism helps for the discovery of new drugs in cancer prevention (Cordero-Herrera et al. 2013). Spinach, Red pepper, Potato, Broccoli, Carrot, Cabbage, Lettuce, Cucumber are the rich source of phytochemicals with antioxidant nature which are indirectly helpful in the anti-proliferation and anti-cancer activity (Houston 2010). Tea beverage mixed up with Averrhoa bilimbi L.
leaves gave new flavour to tea and the antioxidants and phenolic content also has been increased (Anggraini et al. 2016). All these plants had a history of traditional usage for many diseases and according to the fossil records, humans were using the plants as their medicine from the mid paleolithic age.

1.2 HYPOTHESIS

The shade dried fruit extract of *Averrhoa bilimbi* L. can be used for the treatment of UC, ascitic lymphoma and colon cancer cell line COLO-205 (Human) due to the presence of therapeutic biologically active phytochemical compounds having antioxidant, immunomodulatory and cytotoxic effects respectively. The mechanism of these potential actions may be by making use of the presence of significant minerals (Nitrogen, Nitrate, Phosphorous, Potassium, Calcium, Magnesium, Sulphur, Zinc, Ferrous, Copper, Manganese, Molybdenum and Boron) along with Vitamin C as mentioned earlier. The active phytochemicals present in shade dried fruits of *Averrhoa bilimbi* L. were analysed in this study so as to identify and correlate their therapeutic potential.

1.3 REVIEW OF LITERATURE

1.3.1 Fruits as Medicine

Fruits are most efficient and rich source of bioactive compounds with natural organic acids, sugars and endowed with magical qualities that can fight against different diseases. Fruits are often the scavengers of free radicals (Antioxidant activity) (Rechkemmer et al. 2001). Fruits play a challenging role in human nutrition as they are the sources of Vitamins (C, B6, A, E, Niacin, and Thiamine), dietary fibre and minerals (Wargovich 2000). Clinical studies support that the consumption of fruits ameliorates age-related diseases
(Michesa et al. 2000). There is clear scientific proof that the regular intake of fruits can prevent cervical, stomach, pancreatic bladder cancers and high dietary intake of fruits prevents 20% of cancers (Lanzotti and Xiao 2014). Increasing the fruits content in normal diet decreases high blood pressure in humans and thus can protect from the heart diseases (Chobanian et al. 2003).

1.3.2 *Averrhoa* species and its Pharmacological Importance

The different kinds of *Averrhoa* species available in nature are *Averrhoa dolichocarpa*, *Averrhoa leucopetala*, *Averrhoa microphylla*, *Averrhoa minima*, *Averrhoa sinica*, *Averrhoa carambola*, and *Averrhoa bilimbi* L. The fruits of *Averrhoa bilimbi* L. and *Averrhoa carambola* are edible or can be conserved in sugar or sun dried and stored for further use have been reported to have significant biological activity (Ken Love1 et al. 2011). Due to their sour taste they are used in beverages as well as eaten raw. *Averrhoa carambola* is sweet and sour and thus used in the preparations of concentrated juices (Chau et al. 2004) soft drinks and fruit jams (Abdullah and Cheng 2001). *Averrhoa carambola* and *Averrhoa bilimbi* L. fruits are rich sources of Vitamin C, low in calorie with high antioxidants which are advantageous to humans (Bhaskar and Shantaram 2013). Different parts of *Averrhoa* species are used in ayurvedic medicine like antimalarial, antipyretic, anti-helmenthic, antiscorobic agent and antidote for poison (Gheewala et al. 2012). *Averrhoa carambola* fruits has been used for tooth ache, prevents oral infections and are chewed raw (Mohammedet al. 2011). It fights against diabetes, hypertension and renal disturbances (Stasi et al. 2002). The medicinal effects of *Averrhoa bilimbi* L. fruit is not explored much like *Averrhoa carambola*. 
1.3.3 *Averrhoa bilimbi* L.

1.3.3.1 Ecological distribution of *Averrhoa bilimbi* L.

*Averrhoa bilimbi* L. tree is green long-lived, and grows up to 5-10 m (16 to 33 ft) in height. It has a short trunk dividing into a number of upright branches. It is native to Indonesia, Malaysia and foreign to the Australia, Argentina, Brazil, Cuba, Colombia, Ecuador, Guyana, India, Myanmar, Philippines, Singapore, Sri Lanka, Surinam, Tanzania, Thailand and Tobago (Orwa et al. 2009). The natural habitat of *Averrhoa bilimbi* L. is that, it grows in tropical regions shows cold sensitivity and needs sunlight with seasonally humid climates. For the optimum growth of *Averrhoa bilimbi* L., 2-3 months are needed in dry season with evenly distributed rainfall (Anitha et al. 2011).

1.3.3.2 Medicinal uses of *Averrhoa bilimbi* L.

Traditionally *Averrhoa bilimbi* L. is a medicinal plant used to cure cold, cough, itches, boils, Syphilis, Rheumatism, Whooping cough, Diabetes, and hypertension. (Sabiha et al. 2012). Indonesians use it as a potent folk medicine for the treatment of Diabetes mellitus (Wee Yeow Chin 1992). It was reported that *Averrhoa bilimbi* L. has ethno pharmacological properties such as anti-bacterial, anti-inflammatory, anti-scourbutic, astringent, and postpartum protective properties (Goh et al. 1995). The *Averrhoa bilimbi* L. leaves and fruits showed antimicrobial activity against gram positive bacteria like *Bacillus megaterium*, *Bacillus cereus*, *Bacillus subtilis*, *Staphylococcus aureus*, *Streptococcus agalactiae*, *Sarcina lutea* and gram negative bacteria like *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella typhi*, *Salmonella paratyphi*, *Shigella dysenteriae*, *Vibrio cholera*, *Klebsiella pneumonia*, *Aeromonas hydrophila*. The zone of inhibition was more when compared with the standard Ciprofloxacin (Sreedam et al.2011).
The *Averrhoa bilimbi* L. extract showed antifungal activity by inhibiting the growth of the fungi like *Blastomyces dermatitidis*, *Candida albicans*, *Cryptococcus neoformans*, *Pityrosporum ovale*, *Trichophyton spp*, *Aspergillus flavus* and *Microsporum canis*. The zone of Inhibition was found to be equal to that of standard *Fluconazole* (Bijoy Karonet al. 2011). The administration of *Averrhoa bilimbi* L. leaf extract (125mg/kg) was found to decrease the blood glucose and triglycerides when compared with the standard drug Metformin (500mg/kg). The treatment of *Averrhoa bilimbi* L. (intraperitoneal) fruit extract (125 mg/kg/bw) to Alloxan (150 mg/kgbw by intraperitoneal) induced diabetic rats decreased the blood glucose and triglyceride level by 30-50% and increased the HDL by 60% when compared with the control rats (Pushparaj et al. 2000). Intake of *Averrhoa bilimbi* L. fruit homogenate orally for 15 consecutive days did not show any toxic indication or damage to mice up to the studied dose of 1g/kgb.wt. All the experimental rats were in normal condition without any notable toxicity in rats after receiving upto 5000 mg/kgb.wt of *Averrhoa bilimbi* L. fruit extract even after 28 days and hence the LD$_{50}$ value must be above 5000 mg/kg. However a slight change in Haematological parameters was observed at high doses (Savithri et al. 2009). The fruits of *Averrhoa bilimbi* L. squashed with little salt can be used to treat pimples on the face. *Averrhoa bilimbi* L. fruit raw juice has profitable advantages against bilious colic, hypertension, whooping cough and diabetes (Ong and Nordiana 1999).

In human system liver plays a crucial role in the storage, secretion, detoxification and metabolism of nutrition from our daily diet. The *Averrhoa bilimbi* L. was found to maintain the optimum condition of the liver (Hepatoprotective activity) without any liver tissue damage (Nagmoti et al. 2010). The leaves of the *Averrhoa bilimbi* L. (Methanol extract) of 250 and 500 mg/kg concentration significantly ($P \leq 0.01$) rehabilitated the liver function in
CCl₄ induced rats by protecting from liver damages (Nagmoti et al. 2010). In the same way the fruits (250 mg/kg/bwt and 500 mg/kg bwt) also exhibited the hepatoprotective activity against the acetaminophen-induced liver damage in Wistar rats (Thamizh et al. 2015). The ethanolic extract of the leaves of Averrhoa bilimbi L. augmented the healing (10%) qualities of gingival wounds in rats (Igaa 2012). The fruits of Averrhoa bilimbi L. can be used to maintain the optimum cholesterol level, as the aqueous extract of the fruit (125 mg/kg and 50 mg/kg) were found to significantly lower the lipid content in the HFD fed rats (Duangpen et al. 2010). The leaves extract of the Averrhoa bilimbi L. can be used to treat hypertension as it has turned into antihypertension therapeutic medicine (Winarti and Marwati 2009). The Averrhoa bilimbi L. fruits are rich source of Vitamins like riboflavin, Niacin, Vitamin B1, Vitamin A, Ascorbic acid, Carotene and minerals like Calcium, Iron, Phosphorous (Anitha et al. 2010). The wide information of Averrhoa bilimbi L. explains its antibacterial, antifungal, antidiabetic, antihypertensive and wound healing properties. The fruits of Averrhoa bilimbi L. armed with the different pharmacological assets can be used directly or indirectly against cardiovascular and cancer diseases.

The major phytochemicals in the Averrhoa bilimbi L. are phenols, flavonoids, saponins, taninns and glycosides (Xavier et al. 2013) which are highly associated with cancer treatment. It’s identified that the fruits of Averrhoa bilimbi L. is rich source of Vitamin C, amino acids, potassium ions, cyanidin–3–O–H–D–glucoside, sugars and citric acid (Saravana et al. 2011). The fruits of the Averrhoa bilimbi L. are a rich source of antioxidants and can scavenge the free radicals (Rahman et al. 2014). The abnormal levels of the free radicals pave to the oxidative stress and this is one of the major reasons for the pathophysiology of the UC (Rana et al. 2012). The phytochemicals are rich in vegetables and fruits, these phytochemicals are used in the preparation of
medicine for the treatment of cancer (Xiao 2015). A phytochemical Camptothecin (terpene) present in the *Camptotheca acuminata*, has anti-tumor activity and its derivative Topotecan and Irinotecan has been used to treat different cancers (FDA approved) (Hu et al. 2015). Similarly the *Averrhoa bilimbi* L. fruit extract also may have potent phytochemicals which can act against cancer cells.

### 1.3.4 Inflammatory bowel disease

Inflammatory bowel diseases are a kind of inflammatory stipulation where our own body immune system affects the large and small intestine. The IBD is of two types with Crohn’s Disease and Ulcerative Colitis (UC). However the etiology of this is poorly understood. These idiopathict diseases are mainly caused due to the environmental and genetic factors (Bamias et al. 2005). The major difference between CD and UC is the nature of inflammation and the site of inflammation. From mouth to anus any part can be affected with the inflammation in CD and in UC, the inflammation and ulcers were restricted to the colon and rectum region (Baumgart and Carding 2007). Millions of people all over the world are effected by the IBD characterized by uncontrolled inflammation of the intestinal mucosa and (PMNL) poly morpho nuclear leukocyte infiltration, erythema, oedema, ulceration and lipid mediator release (Bilsborough et al. 2015). Prostaglandins, Leukotrienes and Reactive Oxygen Species (ROS) are involved in the induction and development of these diseases (Pavlick et al. 2002). Critically the individuals with IBD have abnormal function of the mucosal immune response corresponding to the gut flora which leads to inflammation of the bowl (IBD). The pathologic differences between Crohn’s disease and ulcerative colitis is shown in figure: 1.1
1.3.5 Ulcerative Colitis

Ulcerative Colitis is a form of IBD which is a chronic inflammatory disorder and restricted to the intestine to the large intestine. It includes ulcers, sores in the affected region and major symptom of this is diarrhea mixed with blood. The exact cause of UC is poorly understood but, the possible causes reported are genetic factors, environmental factors and free radicals (Danese et al. 2004). Ulceration and inflammation cause discomfort and usual colon lymphoid emptying in which macrophage cells produce local mediators of inflammation called cytokines.
1.3.5.1 Pathophysiology of Ulcerative Colitis

The inflammatory cytokines produced are involved in cell activation, cell growth (Wilson et al. 2007), cell differentiation and in the development of inflammation (Sartor 1994). Accumulation of free radicals like superoxide anion (O$_2^-$) in tissue leads to the production of hydrogen peroxide (H$_2$O$_2$) and this oxidative stress plays a vital role in progression and initiation of ulcerative colitis (Kruidenier and Verspaget 2002). The Cyclooxygenase (COX-2) and inducible Nitric oxide synthase (iNOS) are the major enzymes which are involved in the inflammation and associated disorders such as oncogenesis (Akira and Hajime 2007). The activation of COX-2 and iNOS enzymes leads to perilous inflammation which leads to ulcerative colitis (Sakthivel and Guruvayoorappan 2014).

Interleukin 1 (IL-1) gene and multi-drug resistance (MDR) genes are reported to cause unfavourable clinical condition with severe colitis (Cummings and Jewell 2005). Severity of the ulcerative colitis leads to the colorectal cancer (Eaden et al. 2001). In the first 10 years of the disease out of 1000 patient’s diagnosed with ulcerative colitis, two have developed colorectal cancer. It is estimated that in the next decade it will increase to 7 and in the third decade it will be 12 out of 1000. The cumulative probability is of 2% at 10 years, 8% at 20 years and 18% at 30 years (Cottone et al. 2008). The medication for UC is Colonectomy (the removal of large bowel) through surgery. However during such procedures, the other mucosal membrane may be damaged. Anti-inflammatory agents, corticosteroids and some immunomodulators are also used to treat UC. Even though they are effective in management, they develop side effects like cramps, diarrhoea, abdominal pain, fever and high blood pressure (Xuet al.2004). Due to these unfore seen side
effects, researchers are keen on working with natural products to cure ulcerative colitis without side effects.

1.3.5.2 Existing management of UC

The UC is an uncontrolled inflammation in the colon region and a type of Crohn’s diseases, which is a part of IBD that spreads from distal to colonic segments. Major available treatment for the UC is chemotherapy, intravenous steroids and surgery. The treatment to the UC relates to its severity i.e if the UC is in initial stages, corticosteroids and 5-aminosalicylic acid (5-ASA) can be used. The colorectal bleeding patient has to go for surgery (Meier and Sturm 2011).

1.3.5.3 Plant based treatment for inflammation and UC

Corticosteroids and synthetic drugs lead to many side effects and the surgery affects the normal mucosal layer in the colon region. Different medicinal plants (herbal medicine) are considered as the safest method for the treatment of inflammation and UC without any side effects (Umashankar et al. 2011). Some of the plants used for treatment of ulceration are given in table 1.1

<table>
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<tr>
<th>S.No</th>
<th>Medicinal plants and its common name</th>
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<tr>
<td>1</td>
<td><em>Allophylus serratus Kurz</em> (Tippani)</td>
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<tr>
<td>2</td>
<td><em>Aloe vera</em> (<em>L.</em>) (<em>Aloe</em>)</td>
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<tr>
<td>3</td>
<td><em>Butea frondosa</em> <em>Roxb</em> (Flame of the forest)</td>
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<tr>
<td>4</td>
<td><em>Capsicum annuum</em> <em>L.</em> (<em>Chilli pepper</em>)</td>
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Among the phytochemicals polyphenols are the most prevalent class of metabolites in nature and the distribution of these phenols are almost ubiquitous (Souza et al. 2015). Major biological property of the Phenolics are antioxidant, anti-inflammatory and anti-carcinogenic activity (Park et al. 2001). The anti-inflammatory activity of tannins was found to control esophagitis, gastritis, enteritis, and irritating bowel disorders (Cheng et al. 2002). Curcumin obtained from Curcuma longa (majorly available in the Asian countries) was
reported to have apoptosis induction potency (Sumathi et al. 2017). Resveratrol, a polyphenol present in the grape skin has the property of inhibiting the cell growth (induced cell cycle arrest) (Joe et al. 2002). Quercetin, a flavonoid was reported to have anticancer property by inducing the apoptosis (Matsukawa et al. 1997). Thus the phytochemicals of different plant sources are having wide range of medicinal properties.

1.3.6 Cancer

Growth and function of the normal human body depends on the coordinated regulation of cell proliferation, differentiation and apoptosis. This normal function of the human body is altered when cells divide abnormally without apoptosis, leading to development and growth of tumor tissue called cancer. First documented study of cancer (Oncology) was done in 17th century and after cardiovascular diseases cancer is the second most common disease in the world (Jemal et al. 2007). According to WHO (World Health Organization) 8.2 million deaths are recorded all over the world due to Cancer in the year 2012 (Torre et al. 2015). In India up to 2010 about 979786 patients were diagnosed with the cancer (Imran et al. 2011). According to 2018 global cancer statistics around 18.1 new million cancer cases were identified and 9.2% were treated with colorectal cancer (Freddie et al. 2018). In the past two decades advanced technology was developed to treat cancer and this aided us to discover the cancer genome, transcriptome and proteome in detail. Even though the diagnostic and therapeutic advancements are made, cancer continues to be a major medical concern (Magdalena et al. 2001). These cancer cell transfers from place of origin to other organ tissue and affect organs by a process called metastasis. The deterrence of cancer by the diet derived compounds is more beneficial over drug based medication. National Academy of Sciences of the United States gave guidelines in their report stating the importance of diet and

1.3.6.1 Mechanism of Carcinogenesis

Cells with oxidative damage in genes divides before it get repaired and cause permanent genetic alteration, which is the preliminary step to the carcinogenesis. Mutation/alteration with in the gene occur by the drugs, Smoking, ionizing radiation, chemicals and high fat food. (Steven and Manjunath 2013). Covalently the carcinogen bind to DNA in the nucleus and mitochondria. The endogenous carcinogens like ultraviolet radiation and gamma radiation attaches and leads to severe damage to DNA. These carcinogens mutate the normal cellular genes like tumour suppressor gene and proto-oncogenes and cause uncontrolled cell growth which increase the probability of neoplastic transformation (Stanley 1995). The changes in gene expression by carcinogen leads to the activation of specific signalling pathways and tumour promoters that produces the tumour necrosis factor, interleukins (pro-inflammatory cytokines) and nitric oxide which are involved in the inflammation and carcinogenesis (Fischeret al.1995). This mutagenic change in signalling pathway leads to cellular transformation (Cancer) (Reddy et al. 2003). Once the Cancer cells gained foothold it grows faster than the normal healthy cells and healthy cell cannot compete with cancerous cells due to insufficient supply of nutrients from the blood streams. The basic mechanism of cancer and few causative factors of cancer are represented in figure 1.2.
Figure 1.2 Out line of the cancer causing agents to the human body

The tumour suppressor p53 gene is also known as TP53 gene which encodes for a protein that regulates cell cycle and is very important gene in multicellular organism to fight against cancer. The principal mechanism of action of p53 gene is that it helps in apoptosis, inhibition of angiogenesis and genomic stability. The alteration or genetic changes of p53 gene leads to Cancer (Smith et al. 1995). There are 291 genes reported to cause cancer and ninety percent cancer genes show somatic mutations, twenty percent show germ line mutation and ten percent show both. It’s interesting to note that 50 % of all cancers are due to damage or alterations in p53 gene. Chromosomal translocation which create chimeric gene is most common class of mutation in cancer. Several domains are involved in DNA binding and the protein kinase is the most common domain encoded by cancer genes (Andrew et al. 2004).

1.3.6.2 Cancer therapy and management

Current strategies to treat cancer are chemotherapy, surgery and radiation therapy. Chemotherapy helps in shrinking the cancer growth by cytotoxicity on cancer cells. Surgery help in removing the cancerous tumours
from the patient and in radio therapy the cancer cells are damaged and burnt off by the radiations. But all these treatments have limitations and side effects (Lisa and James 2004). The main problem is that the healthy tissues get damaged by these treatments. Recurrence of cancer and drug resistance is other problems faced in these treatments. The best alteration to overcome these problems is only by treating the cancer cells with the natural products. Plants are the major constituents of the natural resources which can fight against wide varieties of diseases without side effects and gives complete cure (Rui 2004).

1.3.6.3 Lymphoma

The major parts of the immune system are lymphoid cells and myeloid cells. The lymphoid cells include T and B lymphocytes and these lymphocytes protect the body from pathogens and alterations in these defence mechanisms will allow the cells to divide and spread faster (Swerdlow et al. 2008). The mutations in the cells lead to the blood cancers and leads to the lymphatic system malfunctioning. Dalton’s ascetic lymphoma (DAL) is a transplantable T-cell lymphoma, mainly originates in thymus region and spreads to the entire host (Reimer et al. 2009). It is well recognized in vivo model to study the anti-lymphoma activity in mice (Chitra et al. 2009).

1.3.6.4 Colon Cancer

Deregulation of the cell signalling pathways and inhibition of the apoptosis are the major concerns of cancer. Colon cancer is one of the major cancer types reported in different parts of the world. The disturbance in the epithelial cells of the colon and rectum are the major reasons for the colon cancer. One of the major reasons for the colon cancer is the serious condition of
the ulcerative colitis that persists in the colon region (McGuckin et al. 2009). The best way to treat this is herbal medicine, this plant based herbal medicine induce the apoptosis and acts as the antitumor agents (Smith-hall et al. 2012). Clinically the cancer patients use plant derived compounds like Texans, Camptothecins, and Vincaalkaloids for their treatment (Safarzadeh et al. 2014).

1.3.7 Plants based medicine for the cancer treatment

*Bauhinia racemosa* stem bark (Gupta et al. 2004), *Cnidoscolus chayamansa* leaves (Kulathuran pillai et al. 2014), Green tea leaf catechins (Geetha and Santhy 2013), *Cinnamomum* inner stem bark (Ramalingam et al. 2015) *Cissampelos pareira* whole plant (Samuel et al. 2015) are some plants which have shown effective treatment efficiency against Dalton’s Ascites Lymphoma cancer cell line. The phytochemicals Resveratrol, Cinnamaldehyde, and Piperine from Red grapes, Cinnamon, Black pepper respectively have shown anti-proliferative effects on colon cancer (Duessel et al. 2008). Ellagic acid is a polyphenol found in most of the fruits (Raspberries, Strawberries etc.) (Loarca Pina et al. 1998) which acts as a detoxifying agent by binding to carcinogen and further making them inactive (Constantinou et al. 1995). Apigenin, a flavone found to inhibit cancer cell signal transduction by induction of apoptosis. It is the best source for treating human anaplastic thyroid carcinoma for which no effective therapy is available (Yin et al. 1999). Apigenin interferes with the epidermal growth factor cell stimulation and reduces the risk of colorectal cancer by interfering with the cancer cell growth signalling pathway (Richter et al. 1999). Tinospora cordifolia, Curcuma longa L., Zizyphus mauritiana Lam and Ocimum sanctum L., are the medicinal plants which were used to treat the lymphoma (Adhvaryu et al. 2008).
1.3.8 Role of free radicals in Ulcerative colitis and Cancer

Free radicals are the molecules or molecular fragments containing one or more unpaired electrons in atomic or molecular orbits. These unpaired electron(s) gives a considerable degree of reactivity on the normal molecules, which in turn becomes a new free radical leading to the formation of a chain reaction. These free radicals are in turn capable of damaging normal working system of the body by interacting with cellular components such as DNA, proteins, carbohydrates and lipids (Valko et al. 2006). Chemical compounds found in plants having no role in growth, photosynthesis, reproduction, but which indirectly participate in the betterment of the plant are called as secondary metabolites. These secondary metabolites are used as medicines, flavouring agents and recreational drugs. Secondary metabolites such as phenols, flavonoids, alkaloids, tannins and saponins are also called as phytochemicals which are biologically active components (Edeoga et al. 2005). Chemotherapeutic and modern drug therapeutic agents produce serious chronic and delayed toxicities that may cause damage to kidneys, lungs and heart (Nitha et al. 2005). The pleasant attributes of phytochemicals are that they protect humans from the environmental and ingested carcinogens. These phytochemicals enhance the DNA repair mechanism and prevent causing cancer progression and metastasis (Robert et al. 2015) without any side effects.

The oxidative stress created by ROS can result in various diseases and disorders (Chanda and Dave 2009) such as cancer, cardiovascular disease, neural disorders, Alzheimers disease, mild cognitive impairment, Parkinson’s disease, Ulcerative Colitis and atherosclerosis. These free radicals are the basic inducers by lipid peroxidation for the transformation, tumor development and for the formation of malignancy. Alteration in the proteins, genetic mutation induction and post translational modifications are the serious problems created.
by the free radicals, finally leading to inflammation and cancer (Seril et al. 2003). In the biopsy of UC and colectomy samples reactive nitrogen and oxygen were reported to be the major reasons for pathogenesis (Lisa et al. 1996). The levels of nitric oxide and iNOS in colon and serum are the major parameters which are evaluated for the UC and an increased iNOS at the inflammation region correlates the UC (McCafferty et al. 1997). As previously quoted, *Averrhoa bilimbi* L. is a rich source of antioxidant which can scavenge the free radicals and can be used for the treatment of the UC and for the cancer.

**1.4 CONCLUSION FROM THE REVIEW OF LITERATURE**

The given literature review explains the available medication for the UC and cancer along with its adverse side effects, and the need of natural products to cure without any side effects. The plants are the major source for the natural drugs which are in the domain of intense research for novel phytochemicals having therapeutic effects. By considering the nutritious nature, phyto constituents, earlier scientific findings on curative effects and traditional uses of the fruits of *Averrhoa bilimbi* L., we intended in this study to analyse the phyto constituents of shade dried methnolic extract of it and to evaluate its medicinal value in treating UC, lymphoma and colon cancer. This study will explore the medicinal significance of *Averrhoa bilimbi* L. fruit extract and the need for further study on its isolated phytochemicals to develop it as an Ayurvedic medicine in future.
1.5 THE OBJECTIVES

- To investigate the phytochemicals of *Averrhoa bilimbi* L. fruit through preliminary tests, quantification of phenolic content and GC-MS analysis.
- To study the effect of *Averrhoa bilimbi* L.fruit extract against acetic acid induced ulcerative colitis in Wistar rats.
- To study the protective effect of *Averrhoa bilimbi* L. fruit extract against Ascitic Lymphoma development in swiss albino mice.
- To analyse the treatment effect of *Averrhoa bilimbi* L. fruit extract on human colon cancer COLO-205 cell line.
- To investigate the *Averrhoa bilimbi* L. fruit extract for its antioxidant activity *in vivo* and *in vitro*. 