CHAPTER 4

STUDY OF ANTI-LYMPHOMA ACTIVITY OF
AVERRHOA BILIMBI L. FRUIT EXTRACT IN SWISS
ALBINO MICE

4.1 INTRODUCTION

In cancer the tumor mass increases rapidly, spreads throughout the body and eventually cause death of the organism (Margarita et al. 2013). Inflammation in the tissue leads to infiltration of the leucocyte, chronic infiltration and this severity leads to the abnormal behaviour of the cells leading to the uncontrolled division of cells escaping apoptosis (Seth rakoff nahoum 2006). In the inflammatory region if the condition is chronic, the alteration of the chemokines, cytokines and increase in the hematopoietic cells at the inflamed tissue leads to carcinogenesis (Fox and Wang 2007). It is thus understood that the inflammation and cancer development are closely associated and the anti-inflammatory agents may have therapeutic effects on cancer cells. In this study we have evaluated the treatment effect of Averrhoa bilimbi L. fruit extract on DAL cell line and in DAL induced mice.

4.2 REASON FOR STUDY

The UC involve the active participation of immune system including lymphatic system. Study reports have shown an association between UC, and
lymphoma (Farrell et al. 2000). Hence we intended to study the anti-lymphoma activity in vivo of *Averrhoa bilimbi* L. fruit extract as it controlled UC in our study. Already we have identified certain biological useful compounds in *Averrhoa bilimbi* L. fruit extract i.e., Hexadecanoic acid (antioxidant activity), and squalene (anticancer property). It was reported that phytochemicals majorly phenolic compounds were found to have both anti-inflammatory and anti-cancer effects (Samuel et al. 2015). Since, *Averrhoa bilimbi* L. fruit is rich in polyphenols and have promising protective effect over UC; it can be used to treat cancer cells. The serious research has been done on the same genus fruit *Averrhoa carambola* (Saghir et al. 2012) and the reports says that *Averrhoa carambola* fruit can be used against cancer, which reduced the hepatocellular carcinoma in mice (Singh et al. 2014). The fruit of the *Averrhoa bilimbi* L. increase the fibroblasts and has the ability to treat gingival wounds in male wistar rats (Hartini 2012).

Dalton’s ascitic lymphoma (DAL) is an inflammatory cancer causing cells, which increase the tumor cells in the peritoneal cavity and the body of the organism increases to maximum (tumor increase) and can cause death. As of now, no in vivo antitumor study on *Averrhoa bilimbi* L. fruit extract against DAL-induced ascitic tumor has been reported. Based on the previous phytochemical study and cytotoxicity activity of fruit extracts, the current investigation of *Averrhoa bilimbi* L. fruit extract against DAL-induced Ascitic tumor in Swiss albino mice is designed. In this study we have analysed the in vitro cytotoxic effects of *Averrhoa bilimbi* L. fruit extract on DAL cell line by MTT assay. We also intended to study the therapeutic and preventive effect of *Averrhoa bilimbi* L. fruit extract on the inflammatory Daltons ascitic lymphoma (DAL) cancer.
4.3 HYPOTHESIS

It is evident that the *Averrhoa bilimbi* L. is a rich source of phytochemicals majorly phenolic compounds with antioxidant nature. In previous chapter it has proven that the *Averrhoa bilimbi* L. fruit decreased the activity of UC which is related to inflammation. We propose that the *Averrhoa bilimbi* L. fruit extract may have a good immune cell regulation activity *in vivo* as it has effectively controlled the UC development upon treatment. Further more, phenolic compounds have been reported to have anti-lymphoma activity (Madumati et al. 2002). Hence we carried out this study to find the treatment effect of *Averrhoa bilimbi* L. fruit against the Lymphoma.

4.4 MATERIALS AND METHODS

4.4.1 Study sample

The shade dried fruits of the plants were subjected for mechanical size reduction. The powdered material 25 gms was extracted with methanol by using soxhlet apparatus and it was concentrated using vacuum rotary evaporator. The obtained extract was preserved in the freezer for further use.

4.4.2 MTT assay for *in vitro* anti-lymphoma study

MTT assay is based on colorimetric assay, in which the potent nature of mitochondrial succinate dehydrogenase enzyme in living cells reduce the yellow soluble substrate MTT (3-(4,5-dimethylthiazol-2yl)-2,5-diphenyltetrazolium bromide ) to formazan which is an insoluble and colored substance. About 0.1 mL of the maintained ascitic fluid (DAL cell line) was collected and diluted with PBS. Cells are seeded at a density of $1.5 \times 10^6$
cells/well (100 μL) in a 96 well plate and incubated in CO₂ incubator at 37°C. After 24 h of incubation, *Averrhoa bilimbi* L. fruit extract in distilled water was added at different concentrations (50, 100, 150, 200, and 250 μg) and incubated for 48 h. Then the MTT solution (0.5 mg/mL) was added to each well and incubated for 4h at 37°C. Immediately, the absorbance of the solution was measured spectrophotometrically at 590 nm using ELISA microplate reader. All the experiments were done in triplicate for each concentration and the mean values were taken. The percentage of viable cells was calculated as per the following formula (Prethi et al. 2012). From this, the percent growth inhibition of DAL cell line was calculated.

\[
\text{Percent viability (\%) = OD of the control (untreated cells)−OD of the test/OD of the control (untreated cells)} \times 100.
\]

From the percent viability, the percent inhibition was calculated as

\[
\% \text{ cell inhibition= } 100 - \% \text{ Viability}
\]

4.4.3 Animals for *in vivo* anti-lymphoma study

Swiss albino male mice of 25 ± 2gm were obtained from Sri Venkateshwara Enterprises, Bangalore, Karnataka, India. Mice were placed in polyacrylic cages (six mice per cage) and housed under standard laboratory conditions with 25°C ± 2°C. Light and dark cycle was maintained as 12:12h. Animals were maintained with free access of standard dry pellet diet from Sri Venkateshwara Enterprises, Bangalore, Karnataka, India, and water *ad libitum*. For 15 days, the mice were acclimatized to laboratory conditions. All the animal experiments were carried out with proper approval from the Institute Animal Ethical Committee (IAEC) of Karunya Institute of Technology and Sciences (IAEC/KU/BT/15/08).
4.4.4 Drugs and chemicals

Gum acacia and ethylenediaminetetraacetic acid (EDTA) was purchased from Hi-Media (Mumbai, India); Drabkin’s solution from Nice Chemicals Pvt. Ltd. (Cochin, India); Standard Drug Methotrexate was obtained from the IPCA laboratories. All other chemicals used were of analytical reagent grade.

4.4.5 Cell line

The DAL cell line was obtained from the National Centre for Cell Science (Pune, India). The concentration of cells was determined using a hemocytometer before transplantation. The DAL cell lines were sustained in mice in vivo by intraperitoneal injection (1.5 × 10^6 cells/mice). After 10-15 days (during Log phase), the ascitic fluid was drawn out from tumor bearing mouse and was injected via i.p. to the experimental mice for induction of ascetic tumor in mice.

4.4.6 In vivo DAL-induced ascitic tumor development and treatment

The solid inflammatory tumor was induced in to the swiss albino mice by injecting the 1.5×10^6 cells/mouse intramuscularly to right hind limb which develop ascitic tumor in 15 days (Sakthivel et al. 2012). The in vivo anti-lymphoma effects were assessed by evaluating the mice body weight, ascitic tumor volume, its weight and the haematological parameters as per the literature (Gupta et al. 2004). During DAL cell line induction metabolic changes and cell damages occur in liver tissue and hence we have analysed the liver histology to evaluate the pathological changes.
The Swiss albino mice were divided into five groups containing 5 mice in each group. Group I served as normal and received vehicle phosphate-buffered saline (PBS) alone. The tumor was induced in group II to V by injecting DAL cell lines \((1.5 \times 10^6 \text{ cells/mouse})\) in PBS intraperitoneal to the left side of mice. Group II served as tumor control. Groups III and IV were treated on second day with *Averrhoa bilimbi* L. fruit extract at doses of 10 mg/kg.bw and 20 mg/kg.bw, respectively (Ambili et al. 2009) Group V was treated with standard drug methotrexate at a dose of 2.5 mg/kg b.wt (Sakthivel et al. 2012). All the treatments were given via i.p. injection at 24th h after DAL tumor cell inoculation and continued for 14 consecutive days.

### 4.4.7 *In vivo* anti-lymphoma study

After 14th day animals were kept under starvation for 1 day and on 15th day after DAL cell line induction and treatments as given above, all mice were weighed and sacrificed for tumor evaluation. Hematological parameters such as red blood cells count (RBC), white blood cells (WBC) count, and haemoglobin (Hb) content were estimated. The antilymphoma was assessed from the body weight of mice and the tumor volume (Chitra et al. 2009). The liver tissue (400 to 500mg) was cut and removed from mice and the pathologic effects in liver tissue were analysed (Kulathuran et al. 2012).

#### 4.4.7.1 Body weight

Weight of the mice was measured in the control group and treated groups before the start of the experiment and after 15 days of treatment. The weights of the normal group of mice also were noted on day one after 15 days of experiments.
4.4.7.2 Tumor volume and weight

On 15th day (completion of experiment), all the animals were sacrificed, and the ascitic fluid was collected from the peritoneal cavity of group II – V mice using syringe after sacrifice. The ascitic fluid was collected in centrifuge tubes, weighed and the volumes were also measured.

4.4.7.3 Haematological parameters

On 15th day of treatment, the animals were sacrificed and the blood was collected through cardiac puncture. The blood was then used for the estimation of total WBC, RBC, and Hb content by following standard methods. Briefly 1:20 for WBC; 1:200 for RBC diluted blood (50µL) was placed over the Neubauer hemocytometer and cells were counted in chamber separately. The cells were counted as;

\[
\text{Cells/ \muL} = \frac{\text{No of cells in 1 large square} \times \text{Dilution factor}}{\text{volume factor (0.1)}}
\]

\[
\text{Dilution factor} = \frac{1}{\text{dilution (20)}}
\]

\[
\text{Volume factor} = (\text{width x length x height}) = 0.1
\]

Haemoglobin was estimated by its colour intensity by colorimetric method (due to its iron), HCl was mixed with the blood in the haemoglobinometer. The Hb is converted to hematin acid, which is identified by the appearance of brown colour. By the comparator box the concentration of the Hb is read directly (Desta et al. 2011).

4.4.7.4 Histopathological evaluation of liver tissue

The histopathologic changes of the liver tissue section of all the experimental mice were analysed. The liver tissue was fixed with formalin
(10%) for 24 hours and then infiltrated and embedded in paraffin. Using microtome fine sections were made and stained with the Hematoxylin and Eosin as per the standard protocol. The stained tissue was imaged under light microscope at different magnification (Desta et al. 2011).

4.5 STATISTICAL ANALYSIS

Experiments were carried out in triplicate and all the values are expressed as mean ± SD. One way analysis was used (ANOVA) i.e., Dunnets square test was used for the statistical analysis. 3.0 version of graph pad software was used and the P values (i.e., *P ≤ 0.05, **P ≤ 0.01, ***P ≤ 0.001) are considered statistically significant compared to DAL tumor control.

4.6 RESULTS

4.6.1 MTT Assay

For increasing concentrations (50µg, 100µg, 150µg, 200µg, 250µg) of Averrhoa bilimbi L. fruit extract, an increased inhibition on DAL cell line growth was observed. All the resulted OD values are taken in triplicate and mean values were used in calculation. The percentage of inhibition and percent of viable cells are given in the table 4.1 and Figure 2. The lower concentration of 50 µg itself showed inhibition of 96.45% ± 0.002 in vitro.
Table 4.1 Percentage of inhibition for the *Averrhoa bilimbi* L. fruit extract DAL cell line

<table>
<thead>
<tr>
<th>Concentration</th>
<th>Percentage of Inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>50µg</td>
<td>96.45 ± 0.02</td>
</tr>
<tr>
<td>100µg</td>
<td>96.98 ± 0.03</td>
</tr>
<tr>
<td>150µg</td>
<td>97.42 ± 0.02</td>
</tr>
<tr>
<td>200µg</td>
<td>97.69 ± 0.01</td>
</tr>
<tr>
<td>250µg</td>
<td>97.96 ± 0.01</td>
</tr>
</tbody>
</table>

Figure 4.1 The percentage of Inhibition of *Averrhoa bilimbi* L. fruit extract on DAL cell line

The IC50 value of the *Averrhoa bilimbi* L. on DAL cell line is 97.42 ± 0.02
4.6.2 Effect of *Averrhoa bilimbi* L. on body weight:

Weight of the mice in control group was increased drastically and the increase is highly significant (**p≤0.0001**) when compared with increase in normal group whereas the weights increase in group III and IV were lower when compared with control group. It clearly shows the therapeutic effect of *Averrhoa bilimbi* L. fruit extract on the DAL induced lymphoma. The difference in body weight of the mice before and after the DAL cell line induction and treatments is as shown in table 4.2. The visual appearance of mice is also shown in Figure 4.3 that indicates the increase in size of mice.

**Table 4.2 Body weight of mice before and after the experiment**

<table>
<thead>
<tr>
<th>Mice</th>
<th>Before DAL cell line Induced to Swiss albino mice in grams (gms)</th>
<th>After DAL cell line Induced to Swiss albino mice in grams (gms) (After 15 days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td>24.66±2.08</td>
<td>25.66±3.51</td>
</tr>
<tr>
<td>Group II</td>
<td>24±1.09</td>
<td>33.33±1.36*a</td>
</tr>
<tr>
<td>Group III</td>
<td>24.83±0.98</td>
<td>27.5±1.22 *b</td>
</tr>
<tr>
<td>Group IV</td>
<td>23.66±1.21</td>
<td>25.66±1.21 *b</td>
</tr>
<tr>
<td>Group V</td>
<td>24.833±1.16</td>
<td>26.5±1.04 *b</td>
</tr>
</tbody>
</table>

The values are represented as mean SD ± *p≤0.05, **p≤0.01, ***p≤0.001 are the indications levels of significance. *a* is the comparison of weight increase in control with normal. *b* is the comparison of group III, IV and V with Control.
Values are expressed in mean ± SD. a control vs normal ***p≤0.0001, b group III vs control **p≤0.001, b group IV vs control ***p≤0.0001, b group V vs control **p≤0.001.

**Figure 4.2 Size of the animal showing difference in the experimental groups**

A. Normal group showing no increase in the body weight; B. Control showing the drastic increase in the body weight due to lymphoma; C. Treatment group with 10mg/kg bwt showing decrease in the body weight due to *Averrhoa bilimbi* L. fruit extract D. Treatment group with 20mg/kgbwt showing decrease in the body weight due to *Averrhoa bilimbi* L. fruit extract (better that group IV) E. Standard drug group V (3.5 mg/kg b.wt) also is showing decrease in the body weight.
4.6.3 Effect of *Averrhoa bilimbi* L. on tumor volume and weight:

The ascitic fluids were collected after 15 days of treatment from peritoneal cavity of group II to V and compared the volume and weights. The *Averrhoa bilimbi* L. fruit extract treatment significantly (p≤0.01) reduced the tumor volume and weight when compared with that of the control group. The tumor volume and tumor weight was also significantly reduced in standard drug treated group.

Table 4.3 Effect of *Averrhoa bilimbi* L. fruit extract on the tumor volume and tumor weight

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Group II</th>
<th>Group III</th>
<th>Group IV</th>
<th>Group V</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tumor Volume in mL</td>
<td>16.16 ± 0.84</td>
<td>11.88 ± 0.63**</td>
<td>6.5 ± 0.73 **</td>
<td>5.42 ± 0.66 **</td>
</tr>
<tr>
<td>Tumor weight in g</td>
<td>15.33 ± 1.15</td>
<td>9.9 ± 0.80 **</td>
<td>6.05 ± 0.62 **</td>
<td>4.22 ± 0.45 **</td>
</tr>
</tbody>
</table>

The treated group showed decreased tumor volume and weight when compared to control group II. All the data are expressed as mean ± *p*≤0.05, **p*≤0.01, ***p*≤0.001 extract treated groups compared with the group II.

4.6.4 Hematological parameters

Total white blood cells (WBC) count was increased in control group when compared with the normal and the WBC count was decreased in group III and IV. For the standard drug also the level of WBC count was decreased. Red blood Cells (RBC) count and hemoglobin percentage were decreased in control group and in Group III and IV they decreased, which is equivalent to the results
of Standard drug treatment. The mean values of RBC count, WBC count and hemoglobin percent are given in table 4.4.

Table 4.4 Total WBC count, RBC and Hemoglobin percentage for the different group of animals

<table>
<thead>
<tr>
<th>Haematological Parameters</th>
<th>Total WBC Count Cells/mL×10⁴</th>
<th>RBC Count 10⁶/µL</th>
<th>Haemoglobin Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td>10.52 ± 1.17</td>
<td>4.83 ± 0.27</td>
<td>13.08 ± 0.83</td>
</tr>
<tr>
<td>Group II</td>
<td>13.9 ± 0.88&lt;sup&gt;a&lt;/sup&gt;&lt;sup&gt;**&lt;/sup&gt;</td>
<td>2.63 ± 0.30&lt;sup&gt;a&lt;/sup&gt;&lt;sup&gt;***&lt;/sup&gt;</td>
<td>5.76 ± 0.47&lt;sup&gt;a&lt;/sup&gt;&lt;sup&gt;***&lt;/sup&gt;</td>
</tr>
<tr>
<td>Group III</td>
<td>11.6 ± 0.45&lt;sup&gt;b&lt;/sup&gt;&lt;sup&gt;**&lt;/sup&gt;</td>
<td>3.87 ± 0.06&lt;sup&gt;b&lt;/sup&gt;&lt;sup&gt;**&lt;/sup&gt;</td>
<td>7.7 ± 0.39&lt;sup&gt;b&lt;/sup&gt;&lt;sup&gt;**&lt;/sup&gt;</td>
</tr>
<tr>
<td>Group IV</td>
<td>10.62 ± 0.48&lt;sup&gt;b&lt;/sup&gt;&lt;sup&gt;**&lt;/sup&gt;</td>
<td>3.26 ± 0.13&lt;sup&gt;b&lt;/sup&gt;&lt;sup&gt;**&lt;/sup&gt;</td>
<td>9.3 ± 0.44&lt;sup&gt;b&lt;/sup&gt;&lt;sup&gt;**&lt;/sup&gt;</td>
</tr>
<tr>
<td>Group V</td>
<td>11.35 ± 0.47&lt;sup&gt;b&lt;/sup&gt;&lt;sup&gt;**&lt;/sup&gt;</td>
<td>3.68 ± 0.18&lt;sup&gt;b&lt;/sup&gt;&lt;sup&gt;**&lt;/sup&gt;</td>
<td>9.2 ± 0.45&lt;sup&gt;b&lt;/sup&gt;&lt;sup&gt;**&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

All the data are expressed as mean ±SD. *p≤0.05, **p≤0.01, ***≤0.001 are the indications of levels of statistical significance. <sup>a</sup> is the comparison between group II and group I. <sup>b</sup> is the comparison of the group III, IV, and V to the group II.

4.6.5 Histopathology report on liver tissue

Normal lobular architecture was observed in the normal group with intact central vein and sinusoids, normal portal tracts and preserved hepatocytes. Necrosis of hepatocytes with surrounding fibrosis and inflammation was observed in control group. Mice treated with *Averrhoa bilimbi* L. fruit extract and standard drug have shown reduced hepatocellular damage, vacuole formation and inflammation. Thus it is clearly evident that *Averrhoa bilimbi* L. fruit extract have protective effect against DAL cell line induction in mice.
Figure 4.3 Histopathological changes in the liver due to the Dalton ascetic lymphoma

Group I: Normal: The architecture of the liver is with preserved hepatocytes, normal lobular with the sinusoids and central vein are intact.
Group II: Control: The liver tissue is surrounded by the fibrosis with perivenular inflammation with high rate of necrosis.
Group III and IV: Treated with the *Averrhoa bilimbi* L. fruit extract (10mg/kg.bw and 20mg/kg.bw) showed the decreased rate of necrosis as an indication of the protective nature from hepatotoxicity.
Group V: Treated with the Standard drug methotrexate (2.5 mg/kg.bw) shows the regular arrangement of the central vein and sinusoids and appearance of the normal lobular structure.

4.7 DISCUSSION

The current investigation was carried out to find out the anti-lymphoma activity of *Averrhoa bilimbi* L. fruit extract in DAL induced mice as well as cytotoxicity activity on DAL cell lines *in vitro* by MTT assay. *Averrhoa bilimbi* L. fruit is rich source of vitamins, minerals and acids which are beneficial to human beings (Ashok Kumar et al.2013). Dietary flavonoids can be used as anti-inflammatory agents and these flavonoids are more in the plants which can be used against inflammation. The flavones and flavonols targets the
transduction enzymes which are involved in the cell surface signalling (Chithan et al. 2005). If the regular diet intake includes polyphenols then it may be possible to escape from exogenous and endogenous mutagens which leads to cancer (Fresco et al. 2006). Vitamin (retinol) directly or indirectly involved in the proliferation, differentiation and apoptosis, acts as a biological regulator. The isotretinoin, retinoic acid, etretinate and acitretin were used in the balance maintenance and normal function of the cutaneous T-cell lymphoma (CTCL).

Combination of the vitamins of same kind or different can be used in the treatment of the cancer (Zhang and Duvic 2003). The leaf extract of the *Murraya koenigi* and *Leptadenia reticulate* are rich source of the phytochemicals and these are found to be responsible for decreasing the lymphoma in the swiss albino mice (Muthumani et al. 2009); (Sathiyarayanan et al. 2007). The fruit of *Averrhoa bilimbi* L. have been reported to have more cytotoxic activity than the leaf (Das et al. 2011). The *in vitro* cytotoxicity of DAL cell lines after treatment with *Averrhoa bilimbi* L. fruit extract was measured by the MTT assay was found to be 97.96 percent for the 250μg of the fruit extract in our study.

The body weight of the mice in the *in vivo* anti-lymphoma study was significantly increased in control group in our study when compared with the normal, which indicate the multiplication of DAL cell line in the control group as evidenced by the other studies (Muthumani et al. 2009). The treatment with *Averrhoa bilimbi* L. fruit extract to group III and group IV mice resulted in significant (p<0.01) decrease in body weight when compared with control. DAL cell line forms ascitic fluid (tumor) and spreads in the entire peritoneal cavity which affects the normal function of liver (Koiri et al. 2017). In our study we have observed an increased tumor volume and weight in control which was significantly reduced in treated group. In correspondence to it, the liver histology also has shown necrotic and architecture damage in control.
which was found to be reduced in treated groups. Such types of liver cell
damage have been observed in lymphoma by other studies (Guruva
yoorappan et al. 2010). In the control group an increased level of total WBC count and
decreased level of RBC count were observed. Treatment with *Averrhoa bilimbi*
L. decreased the level of WBC and increased level of RBC count significantly
(**p<0.01**) when compared with the control group. The haemoglobin
percentage in control was reduced due to DAL cell line induction. In treated
groups, the elevated level of haemoglobin percentage was observed in our
study.

Lymphoma is a type of blood cancer where the immune system was
highly damaged due to the DAL cell line (Koiri et al. 2017). Lymphoma
patients shows altered blood parameters like increased white blood cells and
alterations in Haemoglobin with the enlarged liver in the body (Farn Huei Chan
et al. 2009). The lymphoma is the cancer where the immune system was altered
by escaping from apoptosis. The Dalton ascetic lymphoma causes cell
proliferation, where the cells divide continuously leading to increase in body
size and also it affects the liver, thymus and surrounding parts (Sangeetha and
Venkatarathinakumar 2011).

Plant based natural products such as phenols, flavonoids, terpenoids,
saponins and steroids have received considerable attention due to their diverse
pharmacological properties like antioxidant and antitumor activity (Cragg et al.
2005). Antioxidants play an important role in inhibiting and scavenging
radicals, thus providing protection to humans against infection and degenerative
diseases. Chiefly these compounds have a chemopreventive role in cancer
through their effects on signal transduction in cell proliferation and
angiogenesis (DeFeudis et al. 2003). Ellagic acid is a polyphenol found in fruits
(Raspberries, Strawberries etc.) (Loarca Pina et al.1998) which acts as detoxifying agent by binding to carcinogen and make them inactive (Constantinou et al.1995) and it prevents the binding of carcinogens to DNA and reduces the risk of cancer.

A preliminary phytochemical analysis revealed that the Averrhoa bilimbi L.fruit is rich source of phytochemicals (Bijoy Karon et al. 2011) like phenols, flavonoids, alkaloids, saponins. The camptothecin from Camptotheca acuminate, paclitaxel from Taxus brevifolia are few examples for the phytochemicals treated in the cancer. Ellagic acid involved in the proper function of the p53 signalling and NF-kB signalling. Quercetin has the ability to detoxify the toxin which have the potent nature of causing cancer and help in the apoptosis induction process (Margarita et al. 2013). The best natural phytochemical curcumin not only increases the immune stability of the human body, it also decreases the activity of the T regulatory cells and increases the capacity of the T effector cells to eliminate the cancer cells (Bhattacharyya et al. 2010). However the specific phytochemicals present in this extract need to be analysed.