CHAPTER 5

STUDY OF THE CYTOTOXIC ACTIVITIES OF
AVERRRHOA BILIMBI L. FRUIT EXTRACT AGAINST
COLO-205 HUMAN CANCER CELL LINE

5.1 INTRODUCTION

Numerous synthetic drugs are available in the market for combating several ailments, but they are associated with various side effects which ultimately decreases the quality of human life (Kumar et al. 2007). Hence recent studies have been focussed on herbal medications for the better treatment of diseases without producing any severe side effects. Among the different varieties of herbal medicinal plants listed in Indian Ayurvedic pharmacopeia, the *Averrhoa bilimbi* L. is a major plant from which all the parts like bark, leaves, fruits, stems, flowers and roots have been studied for their various medicinal properties (Ashok et al. 2013). Majorly *Averrhoa bilimbi* L. is having anticancer property and the methanolic extract of the fruit showed better anticancer property on breast cancer cells than the methanolic leaf extract (Nair et al. 2016). From 1975 the incidence of colorectal cancer cases increased and it was a sudden increase in year 1996 to 1998. From the year 2008 to 2010 there was only 3 to 4% decrease of colon cancer cases and the incidence of colon cancer is 30 to 40% more in males when compared with the females (Edward et al. 2014). It was recently estimated that the colon cancer will reach 2.2 million new cases and 1.1 million deaths due to colon cancer in 2030 (Arnold et al. 2016). The available medication for the colon cancer is chemotherapy, surgery
and radiation but, all these are associated with harmful side effects like constipation or diarrhoea, temporary or permanent colostomy, fatigue and sexual dysfunction which ultimately decreases the quality of human life (American Cancer Society 2014). *Averrhoa bilimbi* L. fruit is an edible food and the in vivo toxicity study has reported that the administration of methanolic extract of the fruit up to a concentration of 1 g/kg bw did not produce any harmful effects in the mice (Savithri et al. 2009). In this study, we have analysed the therapeutic effects of the methanolic extract of *Averrhoa bilimbi* L. fruit against human Colon Cancer (COLO-205) cell line. The anti-cancer effect was analysed by cytotoxicity level, DNA fragmentation as an indication of apoptosis, level of apoptosis and cell migration level for metastasis.

The plant based non-nutritive phytochemicals, play a major role in the cancer prevention by initiating the apoptosis or reversing the carcinogenesis. The curcumin from the turmeric, capsaicin from chilli peppers, gingerol from ginger, epigallocatechin from soybeans, lycopene from tomatoes, resveratrol from grapes, caffeic acid phenethyl ester from honey, dially sulphide from garlic, indole-3-carbinol from cabbage and sulphoraphane from the broccoli are the potential phytochemicals which are having the anticancer property (Sruh et al. 2003). Oleuropein is polyphenol obtained from the olive oil which prevents peripulmonary and parenchyma lung metastases (Sepporta et al. 2004).

### 5.2 NEED FOR THE STUDY

*Averrhoa bilimbi* L. fruit possesses a rich content of vital minerals such as zinc, selenium, nitrogen, potassium, phosphorous and iron which regulates cell functions (Dangat et al. 2014) and according to the Dietary
Guidelines of America (2010) it is recommended that our daily intake of fruits and vegetables should be one-half of our plate to prevent diseases like cancer. Fruits are rich source of vitamins and minerals with high quantity of antioxidants which are having biological activities like anti-inflammatory, anti-tumor, anti-diabetic property (Slavin & Lloyd 2012). For the healthy body function and better immunity, minerals play a vital role, where zinc, vanadium, selenium and germanium are used for the prevention of cancer. It was suggested that cancer can be controlled at initial stage if minerals are taken properly and regularly (Mankaram sigh et al. 2012). The lower intake of Vitamin D leads to cancer (Feldman et al. 2014). The K-RAS and BRAF are the major mutations which lead to the colorectal cancer. Vitamin C was found to selectively kill the K-RAS, BRAF mutated colon cancer cells (Yun Jihye et al. 2015). Averrhoa bilimbi L. fruit is the rich source of Vitamin-C, other vitamins, minerals and significant phytochemicals. We have reported the presence of high amount of polyphenols and squalene having anti-cancer effects in the phytochemical analysis in this study.

Averrhoa bilimbi L. showed a very good anti-tumor activity against DAL (Dalton’s ascitic lymphoma) cell line in vitro as well as in in vivo induced Swiss albino mice model in our preliminary study. This Averrhoa bilimbi L. fruit extract also exhibited anti-inflammatory activity in ulcerative colitis animal model in our study i.e., the pre- administration of fruit extract reduced the ulcerative colitis in the Wistar rats by inhibiting the i-NOS and COX-2 and also by decreasing the TNF-α, IL6 and IL1β cytokine levels. All these anti-ulcerative properties were found to be better than the standard drug. The ulcerative colitis and colon cancer are reported to be closely associated with each other (Rubin et al. 2012; Eaden et al. 2001). There is about 2.4 fold chances of developing colon cancer due to the severity of the UC and the risk of UC is still more extensive. In UC diagnosed patients more than 1.6% were
treated with the colitis related cancer (CRC) during past 14 years (Jess et al. 2012). Hence we intended to study the anti-cancer effect of *Averrhoa bilimbi* L. fruit extract on human colon cancer cell line (COLO-205) too.

**5.3 HYPOTHESIS**

We propose that the phytochemicals such as Phenols, Alkaloids, Flavonoids, Saponins and Tannins present in *Averrhoa bilimbi* L. may have molecular action on inhibiting the colon cancer cells and may induce the apoptosis by fragmenting the DNA. Particularly the phytochemical reported by us such as squalene, mannitol has been suggested to have anti-cancer and anti-lymphoma properties and hence *Averrhoa bilimbi* L. fruit extract may have therapeutic effect on human colon cancer cells.

**5.4 MATERIALS AND METHODS**

**5.4.1 Chemicals and reagents**

The chemicals used in this current experiments were purchased from the Hi-Media, Sigma and SD Fine Chemicals. All the chemicals and reagents purchased were of analytical and molecular grade.

**5.4.2 Study sample**

The collected *Averrhoa bilimbi* L. fruits were washed and processed to get fine powder as described earlier. Then 25 gm of *Averrhoa bilimbi* L. fruit powder was used for extraction of the phytochemicals by using 250 mL of methanol as solvent in the soxhlet apparatus, dried by using rotary evaporator and the paste obtained was stored in the freezer for this experiment.
5.4.3 Cell Line

Human colon cancer cell line COLO-205 was procured from the National Centre for Cell Science (NCCS) Pune and were grown in RPMI 1640 medium supplemented with 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin at 37 °C and 5% CO₂ atmosphere. Stock was maintained in vented tissue culture flasks in a CO₂ incubator. All the medium and antibiotics used for the cell lines were purchased from Hi-Media.

5.4.4 Cell growth inhibition assays
5.4.4.1 Trypan blue cell viability assay

The viability of the cells was determined by the penetration of trypan blue dye as it penetrates only into the dead cells. The COLO-205 human colon cancer cells at 2 × 10⁵ cells/mL (80% confluency) were seeded in the 12 well ELISA plate. After 24 hours of incubation, different concentrations (50µg, 100µg, 150µg, 200µg, and 250µg) of the Averrhoa bilimbi L. fruit extract were added to cell lines in 12 well ELISA plate and kept for 48 hours. After incubation cells were centrifuged at 2000 rpm for five minutes and the fresh complete medium was added to the pellet along with 0.4% trypan blue. The trypan blue dye penetrates only into dead cells and were stained blue. The viable and non-viable cells were differentiated by the blue colour (non-viable cells) using a Neubauer Haemocytometer under microscope (10x) and the percentage of lethality was calculated (Raja et al. 2005) by the formula,

\[
\text{Percentage of dead cells} = \frac{\text{(No of non-viable cells)}}{\text{(Total number of cells)}} \times 100.
\]
5.4.4.2 MTT Assay for Cytotoxicity

The methanolic extract of *Averrhoa bilimbi* L. fruit was tested for inhibition on COLO-205 cell growth by using MTT colorimetric assay. MTT (3-(4,5-dimethylthiazol-2-yl) 2,5-diphenyltetrazolium bromide) is an yellow tetrazolium dye, which undergo reduction by mitochondrial oxidoreductase enzyme of live cells and forms purple colour insoluble formazan. COLO-205 human colon cancer cells at 2 × 10^5 cells/mL (80% confluence) were seeded in the 96 well plate and incubated at 37 °C and 5% CO₂ for 24 hours. After the incubation period, *Averrhoa bilimbi* L. fruit extract was added at different concentrations (50µg, 100µg, 150µg, 200µg, and 250µg) in 96 well plate and 25µL (5mg/mL) MTT was added to the cells after 48 hours of incubation. The plate was then incubated for 4 hours after which the media was discarded and 100 µL dimethyl sulfoxide (DMSO) was added to each well in order to dissolve the formazan product. The optical density (absorbance) was measured spectrophotometrically at 570 nm using ELISA plate reader. The percentage of the cell viability was calculated (Mirunalini et al. 2011) using formula:

\[
\text{Percentage of Inhibition} = \frac{\text{OD of the control (untreated cells)} - \text{OD of the test}}{\text{OD of the control (untreated cells)}} \times 100
\]  

5.4.5 Apoptosis induction studies
5.4.5.1 DNA fragmentation analysis

COLO-205 cell line treatment DNA isolation and DNA fragmentation of analysis were performed as per a reported study (Arasu et al. 2016) and this fragmentation is an indication of apoptosis. COLO-205 human colon cancer cells 2 × 10^5 cells/mL (80% confluence) were seeded in the 6 well plate for 24 hours. The IC50 concentration of methanolic extract of *Averrhoa bilimbi* L. from MTT assay was added (126.26 µg) to the wells and incubated
for 24 hours. The media from the plates were then centrifuged at 10000 rpm and the obtained pellets were mixed with 0.5mL of cell lysis buffer (Tris-EDTA SDS lysis buffer) and incubated at 60°C for one hour. The pellet with the lysis buffer was again centrifuged at 10000 rpm and the supernatant was shifted to 5M NaCl: isopropanol ice-cold (vol:vol) containing tubes. These tubes were kept overnight at 20°C and after which it was centrifuged at 10000 rpm for 10 minutes. The pellets were then collected and 70% of ethanol (ice cold) was added. Again the solution was centrifuged at 10000 rpm for 15 minutes and the TE buffer solution was added to the obtained DNA in the tubes. The DNA samples were then analysed for breakage and shearing in 1% agarose gel electrophoresis along with the marker DNA. Then the migration of the DNA and its intact or sheared nature were observed in UV trans-illuminator and imaged it.

The DNA fragmentation normally results in increase in UV absorption by hyper chromic effect of nucleotides. Hence the OD values at 260 nm wavelength also indicate the level of DNA fragmentation (breakage) (Teare et al.1997). We have measured the OD values of the DNAs from control and treated cells at 260nm using UV spectrophotometer and the mean values of triplicate assays were used for comparative analysis.

5.4.5.2 Apoptosis (TUNEL) Assay

Major hallmark for the apoptosis is the fragmentation of DNA and the Terminal deoxynucleotidyl transferase dUTP nick ends were labelled with HRP conjugated antibody to find out the fragmentation of the DNA (Monaorangi et al. 2016). Glass coverslips coated with poly-L-lysine (0.1% (Sigma)) were placed in a 6 well plate (submerge the coverslips in the 6 well
plate) for 15 minutes of incubation. The COLO-205 human colon cancer cells at 2 × 10^5 cells/mL (80% confluency) were seeded in the 6 well plate for 24 hours and further protocol was followed as per the manufacturer’s instruction (Click-iT™ TUNEL IHC Detection Kit). Briefly 1X EdU (5-ethynyl-2’-deoxyuridine) solution was added to the cover slips and incubated at room temperature for 30 minutes. Then the cover slips were washed with the 1X PBS at room temperature for 2 minutes and added 2 drops of the 1X Streptavidin-Peroxidase conjugate and incubated at room temperature for 30 minutes in a humidified chamber. The unbound Streptavidin-Peroxidase was removed by washing thrice with PBS buffer for 2 minutes. The 1:20 dilution of the DAB Chromogen in DAB substrate buffer was prepared and added to the cells which acts a counter strain. The cover slips were washed with PBS first and second with deinoised water for imaging. The immuno-stained brown coloured cells were then evaluated under a research microscope at 40X magnification for counting the TUNEL positive cells. The scoring for the apoptosis positive cells was done manually by counting at corner and centre positions for the positive cells and expressed as mean number of positive cells (Zhao et al. 2015).

5.4.6 Cell migration assay by wound scratch method

Cell migration rate in particular incubation period access the level of metastasis spread. For this, if a scratch (wound) is made in the monolayer cells in six well plate by a sterile tip, the cells at the edges tend to move and recover the scratched (wounded) area upon incubation, correlating the metastatic activity of the cancer cells (Pouliot et al. 2013). Briefly, 2 × 10^5 cells/mL (80% confluency) were seeded in the 6 well plate and incubated for 24 hours. The IC50 concentration of *Averrhoa bilimbi* L. fruit extract (126.26 µg) was added in 6 well plate. Then a scratched area was created by using sterile tip on the surface of confluent monolayer cells and the margin of scratch was noted.
(imaged) at 0th hour. The cells were then allowed to grow by incubating at 12 and 24 hours at 5% CO₂. Then the level of movement of the cells was observed under the microscope and assessed the migration level (Hsu et al. 2007).

5.5 STATISTICAL ANALYSIS

All the in vitro studies were done in triplicate samples and the mean ± SD was used for all data and value expression. A one-way analysis of variance (ANOVA) used for Statistical analysis followed by Dunnett’s test by the Graph pad InStat version 3.0 (Graph pad Software, San Diego, CA).

5.6 RESULTS
5.6.1 Cytotoxicity by Trypan blue Assay

The increasing concentrations (50µg, 100µg, 150µg, 200µg, and 250µg) of the Averrhoa bilimbi L. fruit extract showed increasing cell cytotoxicity and the maximum concentration 250µg of Averrhoa bilimbi L. fruit extract showed higher cytotoxicity of 77.03 ± 4.62 percent. (Table 5.1). The IC50 value is 186.21µg

<table>
<thead>
<tr>
<th>S.No</th>
<th>Concentration</th>
<th>% Cell death</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>50 µg</td>
<td>12.96 ± 3.90</td>
</tr>
<tr>
<td>2</td>
<td>100 µg</td>
<td>27.03 ± 2.31</td>
</tr>
<tr>
<td>3</td>
<td>150 µg</td>
<td>46.66 ± 4.44</td>
</tr>
<tr>
<td>4</td>
<td>200 µg</td>
<td>65.7 ± 1.14</td>
</tr>
<tr>
<td>5</td>
<td>250 µg</td>
<td>77.03 ± 4.62</td>
</tr>
</tbody>
</table>
5.6.2 Cell growth inhibition by MTT Assay

The increasing concentration of *Averrhoa bilimbi* L. fruit extract exhibited an increased inhibition on growth of COLO-205 human colon cancer cells (Table 5.2). The percentage of inhibition and linear graph is shown in figure 5.1. The IC50 value of the *Averrhoa bilimbi* L. fruit extract on cancer cell growth inhibition was found to be 126.26 µg.

Table 5.2 Percent inhibition of COLO-205 cell line by the *Averrhoa bilimbi* L. fruit extract

<table>
<thead>
<tr>
<th>S.No</th>
<th>Concentration</th>
<th>% Inhibition</th>
<th>IC50</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>50 µg</td>
<td>20.65 ± 0.07</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>100 µg</td>
<td>52.83 ± 0.02</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>150 µg</td>
<td>62.7 ± 0.01</td>
<td>126.26 µg</td>
</tr>
<tr>
<td>4</td>
<td>200 µg</td>
<td>80.62 ± 0.08</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>250 µg</td>
<td>90.49 ± 0.03</td>
<td></td>
</tr>
</tbody>
</table>
Figure 5.2 Linear graph between different concentrations of *Averrhoa bilimbi* L. Fruit extract and the % inhibition

5.6.3 DNA fragmentation

By treating with 126.26 µg of *Averrhoa bilimbi* L. Fruit extract for 24 hours, the genomic DNA of COLO-205 colon cancer cells was found to be cleaved, yielding the fragments of DNA, as shown in agarose gel electrophoresis (Figure 5.2). The control showed positive intact DNA without any fragmentation in the gel electrophoresis, whereas the treated DNA sample is showing streaking as an indication of fragmentation.

Figure 5.3 DNA isolated from control and treated COLO-205 human colon cancer cells

L1 is markers (1kb); Lane 2 is the control DNA (Showing intact band) and Lane 3 is the treated DNA which is sheared and smeared as an indication of fragmentation.
The OD values of the DNA in control and treated samples taken from UV spectrophotometric reading at 260 nm is shown in table 5.3. The treated DNA has shown an OD value of 0.77 ± 0.03, which is significantly higher (p≤0.001) than the control DNA (0.4 ± 0.01) as an indication of DNA fragmentation.

**Table 5.3 The mean OD values of DNA at 260nm for the control and treated samples**

<table>
<thead>
<tr>
<th>S.No</th>
<th>Samples</th>
<th>Absorbance at 260 nm (OD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Control</td>
<td>0.4 ± 0.1</td>
</tr>
<tr>
<td>2</td>
<td>Treated</td>
<td>0.77 ± 0.03 **</td>
</tr>
</tbody>
</table>

The value is expressed in mean ±SD and compared between control and treatment, **P≤0.001 is considered as highly significant.

**5.6.4 Cancer cell migration**

The number of COLO-205 cells migrated beyond the scratch margin were evaluated to assess the level of metastasis migration. The migration of the cells was inhibited in treated cells when compared with the cell migration level observed in control sample. In control the movement of the cells was more than that of in treated sample indicating the migration inhibitory activity of *Averrhoa bilimbi* L. fruit extract on human colon cancer cell line COLO-205. This may be helpful to prevent metastasis spread of colon cancer cells.
Control explains the movement of the cells in the wounded area (12 and 24 hours) and in the treated group the movement was restricted by the *Averrhoa bilimbi* L. fruit extract in to the wounded area, which explains the inhibition of migration as an indication of anti-metastatic activity of the *Averrhoa bilimbi* L. fruit extract.

5.6.5 Apoptosis level by TUNEL assay

The brown colour stained nuclei of cells were considered as TUNEL positive apoptotic cells. TUNEL assay was carried out to confirm the apoptosis (cell death) induction activity of *Averrhoa bilimbi* L. fruit extract. Negligible TUNEL positive cells (8.25 ± 5.5) were observed in the control (untreated) group. Where as, significant number (p≤0.001) of apoptotic cells were observed in the treated group (71.5 ± 14.6) when compared with the control group. This result confirms that, the DNA fragmentation shown in our study is due to apoptosis induction.

Figure 5.4 Inhibition of the migration of cells in the control and treated samples (20X)
Figure 5.5 Apoptosis in the treated group and cancer control group

(A) Cancer control; The COLO-205 cells are in compact structure with blue colour
(B) The *Averrhoa bilimbi* L. triggered the apoptosis in the treated group as indicated by the TUNEL positive cells (arrow). Nuclei that showed brown colour with fragmented and condensed DNA are considered as apoptotic cells.

5.7 DISCUSSION

Advances in biotechnology and molecular biology are still not confident in curing diseases completely and one such dreadful disease is cancer. 14 million new cancer cases were recorded in 2012 and new cases are expected to rise to 70% over next 2 decades (Ferlay 2013). According to the WHO reports, cancer is the second largest reason for death globally which was responsible for the 8.8 million deaths in 2015 and nearly 1 in 6 death is due to cancer globally (WHO 2018). Vinblastine and vincristine are the natural products derived from plants which are used to treat cancer along with etoposide and teniposide (semi synthetic derivatives of natural product epipodophyllotoxin) (Lee et al. 2005). Clearly scientific proof indicates that the regular consumption of fruits can prevent cervical, stomach, pancreatic, bladder
cancers and a high diet of fruit intake prevents 20% of cancers (Riboli and Norat 2003). The fruit extract of our study plant *Averrhoa bilimbi* L. also has shown the presence of certain phytochemicals such as squalene which has anticancer property (Chinthalapally et al. 1998). In the current study, we used *Averrhoa bilimbi* L. fruit extract for evaluating its therapeutic efficacy against COLO-205 human colon cancer cell line and we observed promising therapeutic effects which can be further exploited as drug for treating colon cancer. In our study the *Averrhoa bilimbi* L fruit extract (250µg) showed 77.03±0.003 percent cytotoxicity (Table 1) against COLO-205 colon cancer cell line in trypan blue staining assay with an IC50 of 186.21µg. The MTT assay also has shown an increase in cancer cell (COLO-205) growth inhibition with increasing concentration of the fruit extract. The IC50 value was found to be 126.26µg in the MTT assay which we have used for anticancer study. The growth inhibition on COLO-205 cells may be due to cytotoxic effect or regulating the cell cycle or by inducing the apoptosis of cancer cells. The phytochemicals reported in this study in our GC-MS data might have inhibited the COLO-205 cells or induced the apoptosis (DNA fragmentation).

During apoptosis, DNA fragmentation is the major process that activates the apoptosis mediating molecules such as death factor receptor and its adaptor, or Ced4/Apaf-1 and cytochrome C. During apoptosis, DNA degradation occurs in the cells due to the action of caspase inhibitors (Nagata 2000). It has been reported that during apoptosis, the DNA fragmentation shows multiple bands or sheared DNA smearing (Arasu et al. 2016) and such DNA fragmentation can lead to increase in UV absorption at 260nm when compared to the intact DNA of cells by hyper chromic effect (Teare et al. 1997). The measurement of OD at 260nm was thus used as an analytical method for assessing the DNA fragmentation in apoptosis or necrosis. When the COLO-205 cells were treated with the IC50 concentration of *Averrhoa*
*bilimbi* L. fruit extract the genomic DNA fragmentation has resulted as an indication of apoptosis induction while the untreated control sample showed an intact DNA in agarose gel. The OD value of the isolated DNA also was found to be significantly (p≤0.001) increased in treated sample when compared to control as an indication of DNA fragmentation. The DNA fragmentation leads to the apoptosis. But, in necrosis of cells due to cytotoxic effects also the DNA degradation occurs that results in DNA shearing or smearing and increases in UV absorption. Hence to differentiate them, this apoptosis was examined by the TUNEL Immuno Histo Chemistry (IHC) detection kit. Our study results have confirmed that a significant level of apoptosis has been induced by the *Averrhoa bilimbi* L. fruits, as it caused genomic DNA fragmentation as well as apoptosis in COLO-205 colon cancer cells as shown by TUNEL positive cells (Figure 5.3). Not only this all the clinical trials and the new anticancer drugs initially tested on DNA fragmentation (Topala tamara 2014).

The primary tumor when turn into the metastatic state, affecting the other parts of the body is the major cause of death. Eliminating the primary tumor is easier than the metastatic tumor, due to its unknown movement and behaviour. This metastatic cells utilizes all the available oxygen and nutrients which were utilized by the normal cells (Zeng et al. 2003). The detachment and migration or movement of cancer cells are the basic event or hallmark of metastasis *in vivo* (Hsu et al. 2007). This metastasis ability of cancer cells (migration) was analysed by the wound scratch assay, where the movement of the cells were restricted by the drug of our interest (Pouliot et al. 2013). The movement of the COLO-205 human colon cancer cells *in vitro* was analysed by the wound scratch assay (ability of the cells to migrate into wounded area) in our study and this indicates the metastasis migration potential of cancer too.
In this current investigation we have shown that the *Averrhoa bilimbi* L. fruits are having anti-cancer property and this may be due to its rich content of minerals and antioxidant levels which can scavenge the free radicals. The treatment of *Averrhoa bilimbi* L. leaf extract was found to reduce 50% of photo-aging in mice skin (decreased malondialdehyde) when compared with irradiated control (Adelina 2012). Many phytochemicals have been reported to have molecular action on cancers. One such phytochemical is apigenin, a flavonoid found in parsley, artichoke, basil, celery and it induces the apoptosis (Wang et al. 1999). Apigenin inhibit cancer cell signal transduction and induce the apoptosis. It is the best source for treating human anaplastic thyroid carcinoma (Yin et al. 1999). The epidermal growth factor signal transduction pathway is an essential component of both cancer cell growth and differentiation. Apigenin interfere with the epidermal growth factor cell stimulation and reduce the risk of colorectal cancer by interfering with the cancer cell growth signalling pathway (Richter et al. 1999).

The phytochemicals previously reported in this plant by us (Suluvoy et al. 2017) have many biological effects like Hexadecanoic acid a palmitic acid with antioxidant (Kumar et al. 2010); squalene a triterpene with anti-colon cancer activity (Chinthalapally et al. 1998); Oleic acid a aliphatic compounds which reduced blood pressure (Teres et al. 2008). This will encourage other researchers to work further on this fruit extract on colon cancer at molecular, pre-clinical and clinical levels and to find out the pharmacologically active compounds from this fruit. This will lead to new ways for designing effective drugs from this fruit for the treatment of colon cancer in future.