ABSTRACT

Objective: The study is aimed at evaluating the anticancer activity of ethanolic extract of leaves of *Amaranthus cruentus* Linn. against colon cancer cell lines on the basis of its indigenous use and constituents.

Method: The ethanolic extract of the leaves of *Amaranthus cruentus* prepared by using the soxhlet extraction method was subjected to phytochemical analysis to detect the plethora of secondary metabolites present. The total flavonoid content was determined using aluminium chloride colorimetric method. The *in vitro* anticancer activity was studied using MTT assay against HCT – 116 (Colon cancer cell line).

The toxicity study against normal Vero cell line was also done to determine the selectivity of the extract for inhibiting cancer cells. 5-Fluorouracil was used as standard as it is the first choice of drug for the treatment of colon cancer.

Results: The ethanolic extract showed presence of variety of secondary metabolites such as flavonoids, alkaloids, phenols, saponins, steroids and proteins. The IC$_{50}$ value of EEAC was 138±4.24 µg/ml and that of 5-fluorouracil was 32.14±1.58 µg/ml against HCT-116. This indicates that the EEAC has cytotoxic effects comparable with 5-Fluorouracil. The IC$_{50}$ value of EEAC was 1777±78.25µg/ml and that of 5-fluorouracil was 134.4±5.30 µg/ml against Vero cell line. This showed that the plant produce lesser cytotoxic damage to normal cells compared to standard 5 – Fluorouracil.

KEYWORDS: MTT assay, Vero cell line, HCT-116, *Amaranthus cruentus*.

INTRODUCTION

Cancer is a life threatening disease worldwide. It is the second leading cause of death in the world next to cardiovascular disease. The World Health Organization (WHO) statistics for
the year 2011 indicates that 7.9 million deaths were due to cancer. The number of cases was slightly higher in male than females. Lung, breast or prostate and colorectal cancer are the most frequent cancers in countries with high or very high HDIs (Human Development Index). HDI is the composite index which includes three dimensions: long and healthy life, level of education and standard of living which are measured by per capita income. In recent years, countries with low or medium HDIs are also witnessing a rise in cancer of breast, colorectum and lung, indicating that these countries are undergoing economic and human development.[1]

The incidence of cancer is increasing every year. More than 60% of the cancer cases occur in Africa, Asia, Central and South America.

According to ICMR, India is estimated to have over 17.3 lakhs new cases of cancer and over 8.8 lakhs deaths will be due to the cancer by 2020, with breast, lung and cervix cancer topping the list. Data also reveals that only 12.5 per cent of patients come for treatment in early stages of the disease.[2] According to the study conducted by ICMR, breast cancer ranks first among females and mouth cancer among males.

Chemotherapy is recommended based on the type, severity and stage of the cancer. There are more than 100 types of cancer. Among these cancers, lung cancer, prostate or breast cancer and colon cancer are ranked in the first three positions currently. The risk of colon cancer is increased due to delayed appearance of symptoms. Though there are several diagnostic methods available for colon cancer, most cases are diagnosed only at the later stage.

According to GLOBOCAN estimation, it has been reported that there are approximately 12.6 million new colon cancer cases and 7.6 million deaths due to this cancer every year. It is the disease pertaining to our food habits and life style. When treated early, colon cancer has a satisfying 5 year control rate of greater than 65-70%. Colon cancer was found to be interlinked with genetical background. Hence there needs to be a clear and careful surveillance of familial history of hereditary syndromes of colon.[3] Chemotherapy available for treating colon cancer renders prolongation of life span but does not aid in cure of disease. 5 - Flurouracil is the first choice of drug for treating colon cancer. Though it is an effective medicine it is also toxic to normal tissue leading to untoward side effects.

The major toxicity precipitated by anticancer drugs includes bone marrow toxicity, GI mucosal damage, nausea, vomiting, alopecia, damage to organs such as gonads and lungs, delayed wound healing, teratogenicity, etc. From the study conducted to determine the
pattern of ADR caused by chemotherapeutic agents, it has been reported that the most common ADRs were infections (22.4%), nausea/vomiting (21.6%) and febrile neutropenia (13%). The most common drugs precipitating ADR were found to be platinum compounds, nitrogen mustards, taxanes, antibiotics and 5-fluorouracil. The major toxicity is attributed to the ability of anticancer drugs in inhibiting the growth of rapidly dividing normal cells in addition to the tumor cells. Hence, several ongoing researches focus on developing a novel anticancer molecule from various sources with a better safety index.

Several nutraceuticals have also been studied for their efficacy in treating various types of cancer. The curative properties of the plants mainly rely on their secondary metabolites. Many secondary metabolites such as carotenoids, alkaloids, flavonoids and phenols have shown promising cytotoxic activity against various types of cancer. Oxidative stress also results in DNA damage which results in the formation of tumor. Hence antioxidants serve to be other therapeutical option in the treatment of cancer. Many researches have been conducted to discover the effective drug from natural folklore with minimal side effects and selectivity in treating cancer on the basis of their secondary metabolite and antioxidant potential. Dietary supplements are also useful for the prevention and treatment of cancer; they also offer scope for the discovery of novel compound for the treatment of colon cancer.

Amaranthus cruentus L. of amaranthaceae family has been used in indigenous medicine for treating gastric carcinoma and colon cancer. Many of the plants of the genus Amaranthus are widely consumed as part of the normal diet among Indians. They were studied for their cytotoxic effects on different cell lines and animal models, which have shown satisfactory results. Some plants belonging to Amaranthaceae have shown anticancer activity against colon cancer. The leaves of this plant have been reported to possess good antioxidant potential owing to its phytoconstituents. The plant also possesses antibacterial and antifungal activity. However, the cytotoxic potential of the plant against colon cancer has not been validated. Hence this study attempts to evaluate the in vitro anticancer potential of this plant against colon carcinoma using HCT-116 colon cancer cell line.

MATERIALS AND METHODS

Cell lines
HCT- 116 colon cancer cell line and Vero cell line were purchased from National Cancer Center of Science, Pune, India, Minimum essential medium (Hi media, Mumbai, India), 5% Fetal bovine serum (Sigma Aldrich, USA), Penicillin-streptomycin(Sigma Aldrich, USA).

Chemicals
Quercetin, MTT((3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide), Dimethyl sulfoxide (DMSO) and 5 – Fluouracil (standard 5-FU), Reagents were purchased form Sigma Aldrich, USA. All the solvents used for the extraction process are of analytical grade.

i. Plant materials and Preparation of extracts
The plant *Amaranthus cruentus* L. was collected from local market of Mylapore, Chennai in the month of June, 2016. The plant was identified and authenticated by Dr. Sasikala Ethirajalu, Research Officer (Pharmacognosy), Siddha Central Research Institute, Aringar Anna Govt. Hospital Campus, Arumbakkam, Chennai. The leaves of the plant were separated, washed and then shade dried for about a week. The dried leaves were pulverized to a coarse powder by grinding in mixer and stored in an air tight container. 50 g of the powdered leaves of *Amaranthus cruentus* Linn. was weighed and extracted with 500 ml of ethanol wet packed in soxhlet apparatus for about 5 days using soxhlet extraction until the solvent turned colorless in the siphon tube. The extracts were allowed to evaporate. The residue obtained was weighed and stored in desiccator.

ii. Qualitative analysis of extracts
The following qualitative analysis for secondary metabolites was conducted in ethanolic extract of leaves of *Amaranthus cruentus* L(EEAC).\[^9, 10, 11]\n
**Test for flavonoids:** 1 ml of EEAC was taken, to that concentrated hydrochloric acid and magnesium chloride were added and observed for the appearance of tomato red color.

**Test for phenol:** To 1 ml of EEAC, 5 ml of distilled water and few drops of neutral 5% ferric chloride solution were added. A dark green colour indicates the presence of phenolic compounds.

**Test for saponins:** To 1 ml of EEAC, 2 ml of water was added. The solution was shaken in a graduated cylinder for 15 minutes. A layer of foam indicates the presence of saponins.

**Test for tannins:** To 1 ml of EEAC, few drops of 0.1% ferric chloride was added and observed for the development of brownish green or blue black coloration.
Test for alkaloids: To 1 ml of EEAC, few drops of Drangendorff’s reagent was added and allowed to stand for few minutes. A prominent yellow precipitate indicates presence of alkaloids.

Test for protein: To 1 ml of EEAC, few drops of Millon’s reagent was added. A white precipitate indicates the presence of Protein.

Test for steroids: To 1 ml of EEAC, acetic anhydride was added, to that two drops of 10% concentrated sulphuric acid was added and observed for the appearance of green colour.

Test for anthraquinones: To 1 ml of EEAC, 5 ml of 5% solution of ferric chloride and 5 ml dilute hydrochloric acid was added and heated on boiling water-bath for 5 minutes, the solution is cooled and shaken gently with benzene. The organic solvent layer was separated and an equal volume of dilute ammonia was added and observed for change in colour. Pink, red, or violet colour in aqueous layer indicates the presence of anthraquinones.

iii. Quantitative estimation of total flavonoid content
0.5 ml of aliquots of sample (containing 100 µg/ml) and quercetin standard solution of varying concentration (12.5 µg/ml-200 µg/ml) were added into each standard flask of 10 ml capacity containing 4 ml of distilled water. To each flask 0.3 ml of 5% sodium nitrite was added. After 5 mins, 0.3 ml of aluminum chloride was added. After 5 mins, 2ml of sodium hydroxide was added to each flask. The volume was finally made up to 10ml with distilled water and allowed to stand for 15mins at room temperature and filtered. The absorbance of the filtrate was measured at 510nm using UV-Visible spectrophotometer. Blank was performed using distilled water for standard and reagent blank without adding aluminium chloride for recording sample absorbance. All the determinations were done in triplicate. The calibration curve of quercetin standard was plotted with concentration against absorbance. The concentration of the flavonoid present in 50 µg of crude extract is determined using interpolation methods from quercetin standard calibration curve.

From the concentration obtained by interpolation method, the total flavonoid content (TFC) of the extract was determined by using the formula,

$$ TFC = \frac{\text{Concentration} \times \text{Dilution factor} \times \text{volume of stock solution}}{\text{Weight of the extract used}} \times 100 $$
The total flavonoid content of the extracts was expressed as quercetin equivalents (QE) /100 g of dried extract.

iv. In vitro cytotoxic activity by MTT assay
a. Using HCT-116 cell lines
The trypsinized cells from T-25 flask were counted and diluted with MEM medium. HCT-116 cells (1x10^4 cells/well) were seeded in a 96- well plate and incubated for 24 hrs to reach confluency. The cells were then treated with different concentrations of ethanolic leaves of Amaranthus cruentus extracts (6.125, 12.5, 25, 50, 100, 200, 400 and 800 μg/ml) and 5-Fluorouracil (6.125, 12.5, 25, 50, 100, 200, 400 and 800 μg/ml) for 24 hrs. After incubation, MTT solution (5 mg/ ml in phosphate buffered saline) was added to each well and maintained at 37°C in the dark for 4 hr. Finally, 100 μl DMSO (0.1% v/v) was added to dissolve the formazan crystals. The absorbance of each well was measured at a wavelength of 560 nm by ELISA reader.[18, 19,20]

b. Using Vero cell lines
The concentration for the extract was chosen based on its IC_{50} value on the HCT-116 colon carcinoma cell line to determine their toxicity against normal cells and also to determine their selectivity index. The MTT assay was repeated by treating EEAC (200, 400, 800, 1600 and 6400 μg/ml) and 5-Flurouracil(6.125, 12.5, 25, 50, 100, 200, 400 and 800 μg/ml) for 24 hrs on normal Vero cell line.[23-25] The cell viability was calculated using the formula.[21,22]

\[
\text{Cell viability (\%)} = \frac{\text{Absorbance in test wells}}{\text{Absorbance in control wells}} \times 100
\]

The IC_{50} (Median inhibitory concentration) of extract for HCT-116 and Vero cell line was determined.

The selectivity index is the ratio of IC_{50} value of normal cell line and the IC_{50} value of cancer cell lines[26] and it is calculated using the formula

\[SI = \frac{\text{IC}_{50} \text{ of normal cells}}{\text{IC}_{50} \text{ of cancer cells}}\]

Higher index indicate that the extract have higher selectivity towards the cancer cells.

STATISTICAL ANALYSIS
All the assays were performed as triplicates. All the values were represented as Mean±SEM. Data was analyzed using one-way ANOVA followed by Dunnett’s test for the comparison of
The statistical tool applied for the analysis was Graph Pad prism software version 7.3. P values < 0.05 were considered to be significant.

RESULTS

i. Percentage yield of extract

The residue obtained using soxhlet extraction method was weighed and the percentage yield of the EEAC was found to be 9.0% w/w.

ii. Qualitative analysis of extracts

The phytochemical analysis was carried out to determine the nature of compound present in the extracts. The phytochemical analysis of EEAC is represented in table 01.

Table 1: Phytochemical studies of the EEAC

<table>
<thead>
<tr>
<th>S. No</th>
<th>Phytochemical Tests</th>
<th>EEAC</th>
<th>S. No</th>
<th>Phytochemical Tests</th>
<th>EEAC</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Flavonoids</td>
<td>+</td>
<td>5</td>
<td>Proteins</td>
<td>+</td>
</tr>
<tr>
<td>2</td>
<td>Saponins</td>
<td>+</td>
<td>6</td>
<td>Steroids</td>
<td>+</td>
</tr>
<tr>
<td>3</td>
<td>Tannins</td>
<td>-</td>
<td>7</td>
<td>Anthroquinones</td>
<td>-</td>
</tr>
<tr>
<td>4</td>
<td>Alkaloids</td>
<td>+</td>
<td>8</td>
<td>Phenols</td>
<td>+</td>
</tr>
</tbody>
</table>

The EEAC showed positive results for phenols, flavonoids, alkaloids, protein, steroids and saponins indicating their presence in the extract.

iii. Quantitative estimation of total flavonoid content

The standard calibration curve of quercetin was prepared using 12.5, 25, 50, 100, 200 µg/ml of quercetin by AlCl₃ method. Their absorbance was measured using UV spectrophotometer and is tabulated in the table 02.

Table 02: Absorbance of Quercetin at different concentration

<table>
<thead>
<tr>
<th>S. No</th>
<th>Concentration of Quercetin (µg/ml)</th>
<th>Mean Absorbance</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>12.5</td>
<td>0.095</td>
</tr>
<tr>
<td>2</td>
<td>25</td>
<td>0.125</td>
</tr>
<tr>
<td>3</td>
<td>50</td>
<td>0.165</td>
</tr>
<tr>
<td>4</td>
<td>100</td>
<td>0.251</td>
</tr>
<tr>
<td>5</td>
<td>200</td>
<td>0.391</td>
</tr>
</tbody>
</table>

All the values are represented as Mean±SEM (n=3). SEM value is very negligible, hence it is excluded.
From the data given in table 02, the calibration curve of the standard- quercetin was plotted with the help of MS Excel. The absorbance increased with increasing concentrations of quercetin. The correlation coefficient ($R^2$) was found to be 0.996 which denotes good linearity in the values.

![Calibration curve of Quercetin](image)

**Fig. 01: Standard calibration curve of Quercetin**

The total flavonoid content present in the EEAC was determined using the calibration curve of standard quercetin. The total flavonoid content in the EEAC was found to be $8.33\pm0.84$ g QE/100 g of extract.

**iv. In vitro cytotoxic activity by MTT assay**

**a. Cytotoxic effect on HCT-116 by MTT assay**

The percentage viability of the HCT-116 cells on incubation with increasing concentrations of EEAC and 5-Fluorouracil are given in **table 03**.

**Table 03: Cytotoxic effect of EEAC on HCT-116 by MTT assay.**

<table>
<thead>
<tr>
<th>S. No</th>
<th>Concentration (µg/ml)</th>
<th>Percentage cell viability Mean ± SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>EEAC</td>
<td>5-Fluorouracil</td>
</tr>
<tr>
<td>1</td>
<td>Control</td>
<td>100±0</td>
</tr>
<tr>
<td>2</td>
<td>6.25</td>
<td>90.76±0.07</td>
</tr>
<tr>
<td>3</td>
<td>12.5</td>
<td>81.95±0.06</td>
</tr>
<tr>
<td>4</td>
<td>25</td>
<td>72.09±0.10</td>
</tr>
<tr>
<td>5</td>
<td>50</td>
<td>65.41±0.09</td>
</tr>
<tr>
<td>6</td>
<td>100</td>
<td>53.94±0.12</td>
</tr>
<tr>
<td>7</td>
<td>200</td>
<td>42.52±0.08</td>
</tr>
</tbody>
</table>
The IC$_{50}$ values for EEAC and 5-Fluorouracil on HCT-116 cell line calculated using GraphPad Prism Software Version 7.3. The IC$_{50}$ values are represented in table 04.

### Table 04: IC$_{50}$ value of EEAC and 5-Fluorouracil on HCT-116.

<table>
<thead>
<tr>
<th>S. No</th>
<th>Sample</th>
<th>HCT-116 (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Ethanol extract</td>
<td>138±4.25***</td>
</tr>
<tr>
<td>2</td>
<td>5-Flourouracil</td>
<td>32.14±1.58</td>
</tr>
</tbody>
</table>

All the values are represented as Mean±SEM (n=3). *** denotes P values <0.001 in comparison with 5-Flourouracil.

The IC$_{50}$ value of EEAC was 138±4.24 µg/ml and that of 5-fluorouracil was 32.14±1.58 µg/ml. Though the IC$_{50}$ value of EEAC was higher than that of 5-Flourouracil but it is still comparable.

### b. Cytotoxic effect on Vero cell line by MTT assay

The percentage viability of the Vero cells on incubation with increasing concentrations of EEAC and 5-Flourouracil are given in table 05 and table 06.

### Table 05: Cytotoxic effect of Ethanolic extract on Vero cell line by MTT assay.

<table>
<thead>
<tr>
<th>S. No</th>
<th>Concentration (µg/ml)</th>
<th>Percentage cell viability ± SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Control</td>
<td>100±0.00</td>
</tr>
<tr>
<td>2</td>
<td>200</td>
<td>75.21±0.08</td>
</tr>
<tr>
<td>3</td>
<td>400</td>
<td>69.46±0.18</td>
</tr>
<tr>
<td>4</td>
<td>800</td>
<td>62.17±0.16</td>
</tr>
<tr>
<td>5</td>
<td>1600</td>
<td>55.95±0.09</td>
</tr>
<tr>
<td>6</td>
<td>3200</td>
<td>40.55±0.10</td>
</tr>
<tr>
<td>7</td>
<td>6400</td>
<td>30.65±0.18</td>
</tr>
</tbody>
</table>

### Table 06: Cytotoxic effect of 5-flourouracil on Vero cell line using MTT assay.

<table>
<thead>
<tr>
<th>S.No</th>
<th>Concentration (µg/ml)</th>
<th>Percentage cell viability</th>
</tr>
</thead>
</table>

All the values are represented as Mean±SEM (n=3).
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All the values are represented as Mean±SEM (n=3).

The IC$_{50}$ values are calculated for EEAC and 5-Fluorouracil on Vero cell line and tabulated in table 07.

**Table 07: IC$_{50}$ value of EEAC and 5-Fluorouracil on Vero cell line.**

<table>
<thead>
<tr>
<th>S. No</th>
<th>Sample</th>
<th>HCT-116 (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Ethanol extract</td>
<td>1777±78.25***</td>
</tr>
<tr>
<td>2</td>
<td>5-Flurouracil</td>
<td>134.4±5.30</td>
</tr>
</tbody>
</table>

All the values are represented as Mean±SEM (n=3). *** denotes P values <0.001 in comparison with 5-Fluorouracil.

The IC$_{50}$ value of EEAC was 1777±78.25 µg/ml and that of 5-fluorouracil was 134.4±5.30 µg/ml. The IC$_{50}$ value of EEAC was higher than that of 5-Fluorouracil indicating that the EEAC is much less toxic against normal cells.

c. Selectivity index

The selectivity index was calculated and the indices are tabulated as shown in table no. 08.

**Table No. 08: Selectivity index of the Extracts of Amaranthus cruentus L. on HCT-116.**

<table>
<thead>
<tr>
<th>S. No</th>
<th>Sample</th>
<th>Selectivity index (SI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Ethanolic extract</td>
<td>12.88</td>
</tr>
<tr>
<td>2</td>
<td>5-Flurouracil</td>
<td>4.18</td>
</tr>
</tbody>
</table>

The selectivity index for the EEAC was much higher than that of the 5-Fluorouracil. This indicates that the EEAC is more specific for cancer cells than the normal Vero cells.
DISCUSSION

Research on plants as anticancer agents has escalated owing to their indigenous role in treating cancer. Many secondary metabolites have been studied for their cytotoxic potential in treating various cancer conditions. Edible plants are rich in nutritive value. The nutritive values of the plants are generally attributed to a plethora of secondary metabolites. There is ample evidence that the secondary metabolites have beneficial role in preventing and treating diversified clinical conditions. On the basis of its traditional use, presence of secondary metabolites and their antioxidant potential, the *Amaranthus cruentus* L. was chosen for the study. The plant was extracted using ethanol which is the commonly used solvents to extract flavonoids. Previous studies using plants have shown that flavonoids are extracted more efficiently in ethyl acetate and alcohol. It has also been reported that the method of extraction plays a vital role in extraction of desirable phyto-constituents. Laghari et al. (2012) have reported that flavonoids are best extracted by soxhlet method of extraction or microwave extraction using ethanol or ethyl acetate as solvents.[30] Hence in this study the soxhlet method of extraction using ethanol was used.

It has been shown that the secondary metabolites such as flavonoids, phenols, alkaloids and protein have significant role in the treatment and prevention of colon cancer.[31] The phytochemical analysis revealed that ethanolic extract of leaves of *Amaranthus cruentus* L. contain flavonoids, phenols, saponins, proteins, steroids and alkaloids.

In order to determine the cytotoxic potential of the plant, preliminary *in vitro* study was conducted using MTT assay. The assay is based on the principle that the viable cells contain mitochondrial dehydrogenase which converts the dye into blue coloured formazan dye. The absorbance produced is directly proportional to the number of viable cells. The IC$_{50}$ value is the concentration at which the sample exhibits 50% inhibition of growth of cells.

It is observed that the ethanolic extract had a wide spectrum of secondary metabolites and the concentration of flavonoid was 8.33±0.84 g QE/100 g of extract. From IC$_{50}$ value of EEAC on HCT-116 cell line and vero cell line, it is evident that the EEAC requires 10 fold increase in concentration to be cytotoxic to normal Vero cell line. The 5-FU has shown only a 5 fold increase in concentration to exhibit cytotoxicity to normal vero cell line.
The selectivity index of the EEAC for HCT-116 cell lines is significantly higher when compared with the 5-fluorouracil. This suggests that the EEAC has a more specific action against cancer cell line without being toxic to normal cells.

Several studies have suggested that natural flavonoids have growth inhibitory effects on various kinds of cancer cells. These are known to act on different molecular targets and the actions are mediated by interfering with diverse metabolic pathways. In the case of bladder cancer, flavones apigenin and luteolin seem to be cytotoxicity most active. Besides these two flavones, chrysin and the flavonol kaempferol have also been reported to have anti-proliferative activity and induce apoptosis in oral cavity cancer cells. Yang et al. (2008), have reported in this study that flavonoids may be selective for a particular type of cancer and may not show activity against other types of cancer.\[32, 33\]

In the present study, it is postulated that the cytotoxic activity of EEAC is directly related to the presence of flavonoids especially Rutin. Studies have shown that rutin plays a beneficial role in inhibiting the growth of tumor. The possible mechanism through which they mediate anticancer effect was suggested to be their ability to induce apoptosis and cell cycle arrest. They also modulate the infiltration of macrophages; reduce the inter-peritoneal colorectal carcinoma metastasis. However, some activity patterns and tissue specificities of flavonoids such as rutin and other metabolites for various organ sites are essential to be explored.\[34\]

**CONCLUSION**

From the study it is concluded that the EEAC was found to possess good cytotoxic activity against HCT-116 cell line. It also has a comparatively higher selectivity index when compared with 5-Fluorouracil. This study has been conducted only as a preclinical testing to evaluate the anticancer activity of EEAC against colon carcinoma. Further studies on their mechanism, isolation of individual compounds and their activity on animal models are required before it can be taken up for clinical studies.

**REFERENCE**


