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Research Article

IN VITRO ANTIPROLIFERATIVE EFFECTS OF ANTHOCYANIN EXTRACTED FROM RED SORGHUM (*SORGHUM BICOLOR*) BRAN ON HUMAN LARYNX CARCINOMA CELL LINE

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ABSTRACT

Tumor metastasis is the most important cause of death due to cancer, hence various treatment strategies have developed and targeted on preventing the occurrence of metastasis. Anthocyanins, a natural colourants are rich in antioxidants belonging to flavanoid family. These anthocyanins are known for their antiproliferative effects. The aim of the present study was to extract the anthocyanin from red sorghum bran by using two solvents methanol and acidified methanol. The anthocyanin extracts were investigated for their potential chemo preventive activity against Human Epithelial larynx cell line (Hep 2). The sorghum anthocyanin act as a most potent inhibitor of Hep 2 cell and its growth was inhibited ~90% after 24 hrs of exposure in acidified methanol extract, illustrating greater growth inhibition of larynx cancer, as compared to methanol extract which inhibited ~ 70% at 1000 µg/ml. Sorghum anthocyanins were 3 - deoxyanthocyanidins with or without glycosylation. The varying compositions and degrees of growth inhibition suggest that the anthocyanin chemical structure may play an important role in the growth inhibitory activity of sorghum anthocyanins. The acidified methanol extracts showed comparably higher activity than methanol extracts. This is assumed that the acidified methanol preserves the 3 - deoxyanthocyanidins in stable condition.

Keywords: Sorghum, Anthocyanin, Hep- 2 cell line, Cell growth.

INTRODUCTION

Cancer is the leading cause of mortality world wide. The failure of conventional chemotherapy to effect major reduction in the mortality indicates that new approaches are critically needed. (Kapadia, 2000). An extremely promising strategy for cancer prevention today is chemoprevention, which is defined as the use of synthetic or natural agents to block the development of cancer in humans. A variety of bioactive compounds and their derivatives have been shown to inhibit carcinogenesis in a number of experimental systems involving initiation, promotion and progression (Ho, *et.al.,* 1994, Huang *et.al.,* 1994).

Anthocyanins, natural pigments present in fruits and vegetables have shown considerable potential in the food industry as safe and effective food colorants. Anthocyanins also possess known pharmacological properties and are used by humans for therapeutic purposes. In sorghum, the most common anthocyanin types are the 3 – deoxyanthocyanidins and their derivatives (Nip and Burns, 1969; Gous 1989). Most of the data were obtained for fruit anthocyanins which are thought to contribute significantly to the health benefits of fruit consumption. We have not found any work reporting biological properties of the 3 - deoxyanthocyanidins commonly found in sorghum. So, the sorghum anthocyanins should be investigated for any unique health properties.

The objective of the present study was to compare the effects of two different solvent extracts of red sorghum anthocyanins against Human Epithelial larynx (Hep 2) cell line.

MATERIALS AND METHODS

Samples

The bran of *Sorghum bicolor* (L.) red sorghum were collected from farmers field in Tamil Nadu, India and were stored at - 20°C.

Anthocyanin extraction

The anthocyanin extraction protocol involved the addition of 10 ml of solvent (1% HCl in methanol) to 0.5 g of sample in 50 ml centrifuge tubes and shaking the samples for 2 h at low speed (75 rpm) in an orbitory shaker (Neolab). Samples were then stored at -20°C for overnight in the dark to allow for maximum diffusion of phenolics from the cellular matrix. Samples were then equilibrated to room temperature and centrifuged at 7,000 rpm for 10 min and taken for analysis. Residues were rinsed with 10 ml volumes of solvent for two times with shaking for 5 min, then centrifuging at 7000 rpm for 10 min and taken for analysis. Finally, the extracts were mixed well and stored at -20°C in the dark until further biochemical analysis. (Joseph *et al.,* 2004)

Cell culture

Human Larynx cancer cell line, Hep 2, cells were maintained in DMEM containing 10% FBS, supplemented with additional glutamine (0.03%) and 100 µg/ml benzyl penicillin, 100 U/ml streptomycin and 2.5 µg/ml amphotericin. Cells were allowed to grow in tissue culture flasks (Corning, USA) and were kept in CO_2 incubator at 37°C in a humidified atmosphere of 5% CO2 and 95% air. For experimental purpose, cells from exponentially growing culture were used. All experiments were repeated three times.

Cytotoxicity assay

The anthocyanin extracts was tested against Hep 2 cell line and incubated for 72 h after which the MTT assay [3-(4, 5-dimethyl thiazol-2yl)-2, 5-diphenyl tetrazolium bromide] was carried out as described by Mossman in 1982. The cytotoxic activity was expressed as mean concentration of the extract required to kill 50% of the cell population (IC_{50}).

RESULTS AND DISCUSSION

Plant pigments such as anthocaynins possess known pharmacological properties and were used by humans for therapeutic purposes. Inhibition of cancer cell growth by the anthocyanins in this study was measured by the MTT assay.

Effects of sorghum anthocaynins on the growth inhibition of Hep – 2 cells.

Invitro antiproliferative activity of anthocaynin was performed by MTT assay. Table -1 shows the effect of anthocaynin on Hep 2 cells extracted from red sorghum bran by using acidified methanol. In our results it was observed that ~90% inhibition of Hep – 2 cell line at 1000 µg/ml after 24 hrs. It was also observed that as the concentration of anthocaynin decreases the cell viability increases. The highest concentration displayed a highest inhibition in a dose dependent antiproliferative activity on Hep – 2 cells (Fig 1). Untreated Hep 2 cells appeared as elongated shape, attached smoothly on the culture cell surface and some of the cells grouped together to form colonies. (Fig 3).

Following treatment with anthocyanin extract for 24 hrs, the cells changed to round shape and lost cell contact. (Fig 4). In particular, the cells changed their surface morphology and died at a concentration of 1000 µg/ml and the cell viability was found to be 11.76. This study also confirms the *invitro* antiproliferative property of sorghum anthocaynin against Hep – 2 cancer cell line. Fig 4 – 6 shows the morphological changes of Hep – 2 cells at different concentration of anthocyanin extracted by using acidified methanol.

Table 2 shows the effect of anthocyanin extracted from red sorghum bran by Hep 2 using methanol. Around ~70% inhibition of Hep – 2 cells was observed at 1000 µg/ml concentration after 24 hrs. Fig 2 indicated the relative cell viability of sorghum anthocyanin at different concentration. Following treatment with anthocyanin extract for 24 hrs, the cells lost their surface morphology and died at a concentration of 1000 µg/ml and cell viability was found to be 29.41%. (Fig 7). Fig 7 – 10 shows the morphological changes of Hep – 2 cells at different concentration of anthocaynin extracted by using methanol.

Liu *et.al.,* 2002 reported the antiproliferative activity of anthocyanin from raseberry extract against Hep G2 cells.

Although there are numerous reports on inhibition of cancer cell growth *invitro* by anthocyanin extracted from various fruits and vegetables (Koide *et.al.,* 1996), to our knowledge no one has reported the effects of sorghum anthocyanins. Hence our study explains the inhibitory effect of Hep 2 cell line by sorghum anthocyanin.

Zhao *et.al.,* 2004 reported that 50% inhibition of anthocyanin extracted from biliberry on HT – 29 cell lines at 25 µg/ml after 24 hrs of exposure. They concluded that although anthocyanins are natural colourants and exist widely in many fruits, flowers and other plant materials, they are not stable pigments. Anthocyanins undergo reversible structural transformations and dramatic changes in colour with changes in pH levels. At a pH of 3, or below the anthocyanin are in stable form. As the pH is raised, the anthocyanin was found to be unstable due to changes in chemical structures by hydration and proton transfer reactions. The increased temperature also plays a role in degradation of anthocyanin.

Sorghum anthocyanins were 3 – deoxy anthocyanidins which were stable at high pH and temperature. The culture conditions used in our study were at pH 7.4 and temperature at 37º C. Since these 3 – deoxy anthocyanidins are stable at these conditions and degradation of anthocyanins did not happen. So, sorghum anthocyanins extracted from acidified methanol showed ~90% inhibition on Hep-2 cells. So the chemical structure plays an important role in inhibitory activity.

To draw reasonable conclusions, the available data on cancer by anthocyanins are too limited. Nip and Burns, (1969) reported luteolinidin and apigenindin the constituents of 3 – deoxy anthocyanidins. Manthey *et.al.,* 2002 reported the strongest antiproliferative activity against Human cancer cell lines by luteolinidin and apigenindin. So the antiproliferative activity sorghum anthocaynin against Hep -2 cells exhibited by luteolinidin and apigenindin.

The search for better cancer chemotherapeutic agents is ongoing all over the world. The present study demonstrates that these sorghum anthocyanins have significant growth inhibitory effects in Human Epithelial larynx carcinoma cells (Hep- 2). This study is the first report about the inhibitory effect of sorghum anthocyanins on Hep – 2 Cells.

In conclusion, these findings demonstrated that the sorghum anthocaynins might exert antitumour activity against Hep – 2 cells. The results of these investigations should be helpful in explaining the complex pharmacological activities. This study also confirmed the sorghum anthocaynins and its potential to inhibit the cancer cells. Further more mechanistic work is essential to prove these compounds as one of the specific cancer drug.

Table 1: Shows the effect of anthocyanin on Hep 2 cells extracted from red sorghum bran by using acidified methanol.

S.no	Concentration (µg/ml)	Dilutions	Absorbance	Cell viability
1	1000	Neat	0.06	11.76
2	500	1:1	0.15	29.41
3	250	1:2	0.19	37.25
4	125	1:4	0.24	47.05
5	62.5	1:8	0.28	54.90
6	31.25	1:16	0.32	62.74
7	15.625	1:32	0.49	96.07
8	Cell control	-	0.51	100

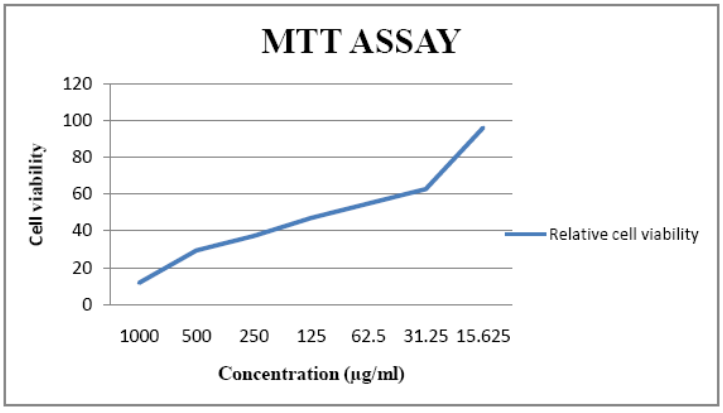

Fig. 1: Shows the relative cell viability of Hep 2 cells at different concentration of anthocaynin extracted by acidified methanol.

Table 2: shows the effect of anthocaynin on Hep 2 cells extracted from red sorghum bran by using methanol.

S.no	Concentration (µg/ml)	Dilutions	Absorbance	Cell viability
1	1000	Neat	0.15	29.41
2	500	1:1	0.17	33.33
3	250	1:2	0.23	45.09
4	125	1:4	0.29	56.86
5	62.5	1:8	0.34	66.66
6	31.25	1:16	0.42	82.35
7	15.625	1:32	0.50	98.03
8	Cell control	-	0.51	100

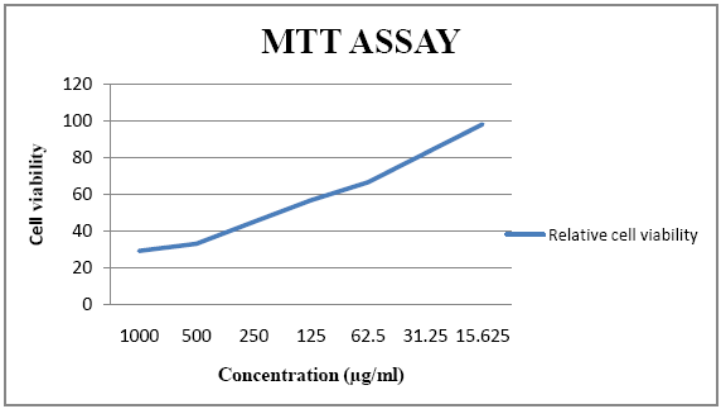

Fig. 2: Shows the relative cell viability of HEP 2 cells at different concentration of anthocaynin extracted by methanol.

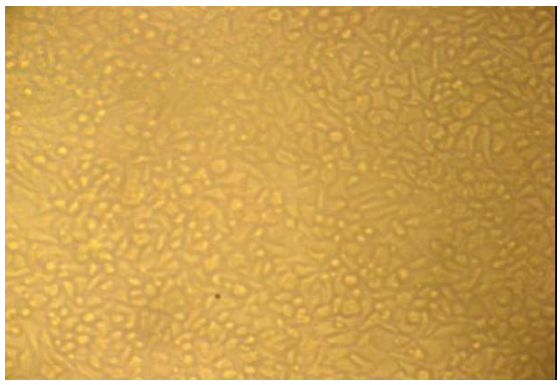

Fig. 3: Normal Hep 2 cells shows oval or rod shaped cells with cell to cell anchorage

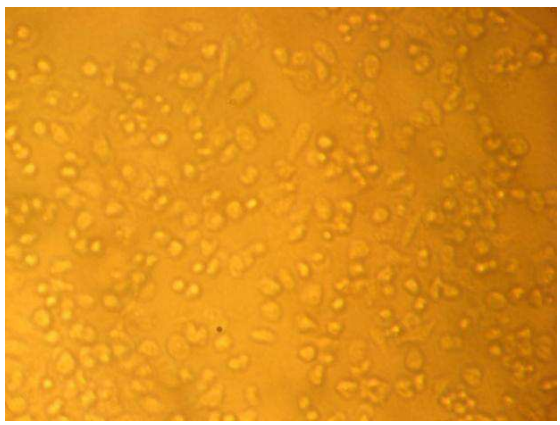

Fig. 4: Acidified methanol extracted anthocyanin treated Hep2 cells shows spherical shaped cells leading to loss of cell anchorage with concentration of 1000µg / ml

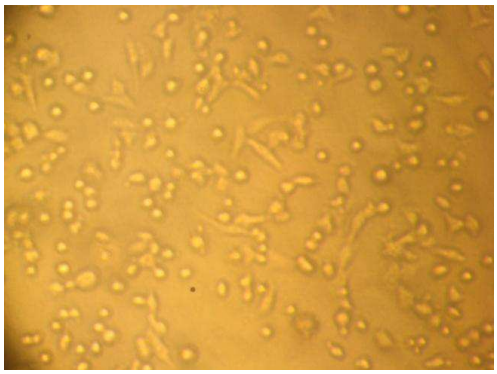

Fig. 5: Acidified methanol extracted anthocyanin treated Hep2 cells shows spherical shaped cells leading to loss of cell anchorage with concentration of 500µg / ml

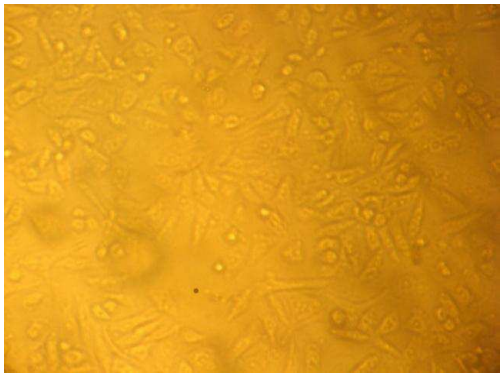

Fig. 6: Acidified methanol extracted anthocyanin treated Hep2 cells shows rod shaped cells leading to loss of cell anchorage with concentration of 250µg / ml (Mild toxicity)

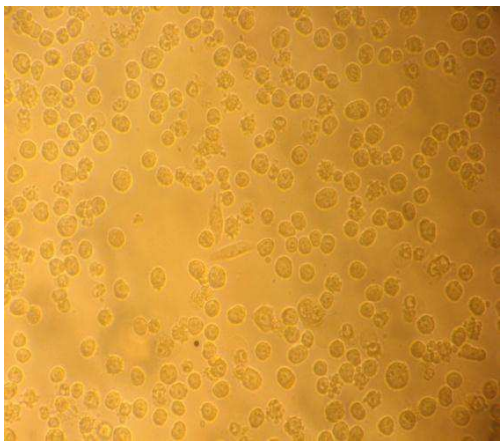

Fig. 7: Methanol extracted anthocyanin treated Hep2 cells shows spherical shaped cells leading to loss of cell anchorage with concentration of1000µg / ml

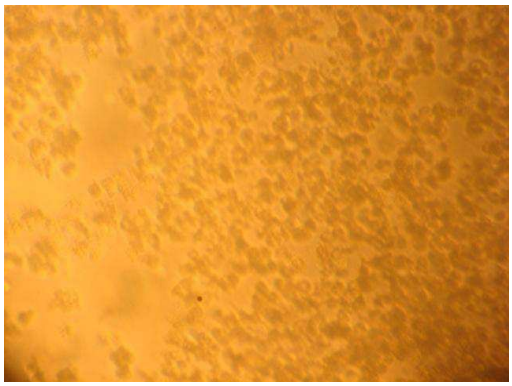

Fig. 8: Methanol extracted anthocyanin treated Hep2 cells shows spherical shaped cells leading to loss of cell anchorage with concentration of 500µg / ml

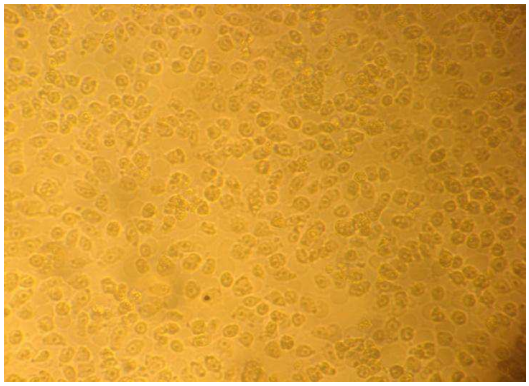

Fig. 9: Acidified methanol extracted anthocyanin treated Hep2 cells shows spherical shaped cells leading to loss of cell anchorage with concentration of 250µg / ml

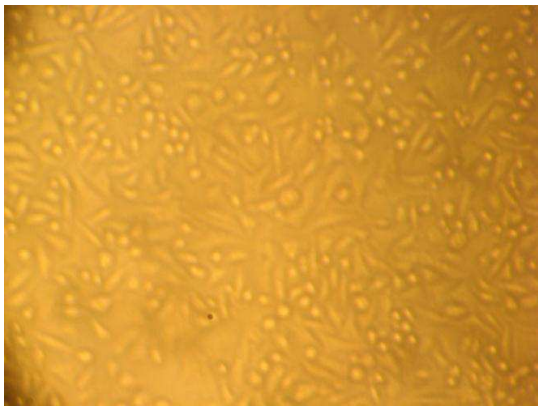

Fig. 10: Methanol extracted anthocyanin treated Hep2 cells shows rod shaped cells leading to loss of cell anchorage with concentration of 125µg / ml

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