

Regular Article

***In vitro* evaluation of anticancer property of anthocyanin extract from *Musa acuminata* bract**

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**Anthocyanins are potent anticarcinogenic properties against several cancers thus demonstrating potential for cancer prevention. In the present study, we have investigated the chemopreventive effects of anthocyanin extracted from *Musa acuminata* bract against human breast cancer cell line (MCF-7). Acidified methanolic extracts from *Musa acuminata* bract has shown 12.24% inhibition at 1000µg/ml concentration against MCF-7 cell line. Results indicated that anthocyanin from *Musa acuminata* bract extracts had strong antiproliferative activity against MCF-7 cell lines at varying concentration. These results encourage the bioactive constituents as chemopreventive agents for human breast cancer.**

Cancer is one of the main causes of death in the world. In 2004, cancer was the second cause of death in the world, just below cardiovascular diseases, with more deaths. In women, breast cancer is the second most important cause of cancer related deaths. It was estimated that in 2007, about 1.4 million new cases of cancer were diagnosed, cancers of the prostate and breast being the most frequently diagnosed in men and women, respectively, followed by lung and colorectal cancers both in men and in women (American Cancer Society, 2007). Cancer, a disease resulting from deregulated cell growth control, is caused by the interaction of dietary, genetic, and environmental risk factors. Dietary factors are considered to play a major role in cancer etiology. It has been estimated that potential for cancer prevention by a healthy diet and lifestyle is excellent and might reduce the burden of frequently occurring cancers of the breast, prostate and colon by 33-55%, 10-20% and 66-75%, respectively (Young *et al.*, 2002). Cancer is ultimately the end stage of a chronic disease process characterized by abnormal cell and tissue differentiation: starts as an increase in the number of abnormal cells derived from a given normal tissue, then invasion of adjacent tissues by these abnormal cells, and lymphatic or blood-borne spread of malignant cells to regional lymph nodes and to distant sites occurs. This process which eventually leads to the final outcome of invasive and metastatic cancer is carcinogenesis (Sporn and Suh, 2000).

Histological, epidemiological and experimental data suggest that breast carcinogenesis starts with hyperplasia, progressing through atypical hyperplasia to *in situ* and invasive carcinoma. The time course of these changes is difficult to estimate because, during this multistep process, unknown factors may stop progression and the hyperplastic lesions may regress and never undergo a malignant transformation (Bai *et al.*, 2001).

Cancer chemoprevention is an approach that has been studied for the last years in order to address the disease. Many natural compounds have been studied; among them phenolic compounds from fruits and vegetables have been extensively studied for their antioxidant properties related to oxidative stress and cancer prevention. Cancer preventive phytochemicals have been shown to suppress or block carcinogenesis by a variety of mechanisms including acting as antioxidants or antiproliferative agents (Singletary *et al.*, 2003). Polyphenols are reducing agents, and together with other dietary reducing agents, referred to as antioxidants, protect the body's tissues against oxidative stress and associated pathologies such as cancers, coronary heart disease and inflammation (Tapiero *et al.*, 2002).

Anthocyanins are natural pigments that provide intense purple to red color in many fruits and vegetables such as blueberries, grapes, red cabbages and purple corn. In recent years, considerable studies have exhibited the ability of anthocyanins to inhibit oxidative stress (Tsuda *et al.*, 2000, Miranda-Rottmann *et al.*, 2002) and to induce apoptosis in malignant cells (Yi *et al.*, 2005; Kuo *et al.*, 2004) which both suggest that anthocyanins may prevent carcinogenesis.

The objective of the present study is to evaluate the potential of *Musa acuminata* bract anthocyanin as inhibitor for breast cancer cell line.

## Materials and Methods

### Cell line and culture

Human breast cancer MCF - 7 (GDC055) cell lines were obtained from National Centre for Cell Sciences, Pune (NCCS). The cells were maintained in RPMI-1640 supplemented with 10% FBS, penicillin (100 U/ml), and streptomycin (100 µg/ml) in a humidified atmosphere of 50 µg/ml CO<sub>2</sub> at 37 °C.

### Reagents

RPMI-1640 was purchased from GIBCO/BRL Invitrogen (Gaithersburg, MD). Fetal bovine serum (FBS) was purchased from Gibco laboratories Trypsin, methylthiazolyl diphenyl- tetrazolium bromide (MTT), and dimethyl sulfoxide (DMSO) were purchased from (Sisco research laboratory chemicals Mumbai). All of other chemicals and reagents were obtained from Sigma Aldrich, Mumbai.

### *In vitro* assay for Cytotoxicity activity (MTT assay)

The Cytotoxicity of samples on MCF-7 cells was determined by the MTT assay (Mosmann *et al.*, 1983). Cells ( $1 \times 10^5$ /well) were plated in 100 µl of medium/well in 96-well plates (Hi media). After 48 hours incubation the cell reaches the confluence. Then, cells were incubated in the presence of various concentrations of the samples in 0.1% DMSO for 48h at 37°C. After removal of the sample solution and washing with phosphate-buffered saline (pH 7.4), 20µl/well (5mg/ml) of 0.5% 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl--tetrazolium bromide cells(MTT) phosphate- buffered saline solution was added. After 4h incubation, 0.04M HCl/ isopropanol were added. Viable cells were determined by the absorbance at 570nm with reference at 655nm. Measurements were performed in 3 times, and the concentration required for a 50% inhibition of viability (IC<sub>50</sub>) was determined graphically. The absorbance at 570 nm was measured with a microplate reader (Bio-Rad, Richmond, CA), using wells without sample containing cells as blanks. All experiments were performed in triplicate. The effect of the samples on the proliferation of human breast cancer cells was expressed as the % cell viability, using the following formula:

$$\% \text{ cell viability} = A_{570} \text{ of treated cells} / A_{570} \text{ of control cells} \times 100\%.$$

## Results and Discussion

### Morphological changes

In the present study, antiproliferative effect of anthocyanin extracted from *Musa acuminata* bract on breast cancer cell line, MCF-7 was investigated under different concentrations. The anthocyanin produced significant morphological alterations on MCF-7 in culture. Under normal growth conditions(control) these cells were regular in shape and size and adhere to substratum (Figure 1a). After treatment with 1000 $\mu$ g of anthocyanin, the cells become irregular in shape and size and causes some changes on the cell surface associated with adhere of the substratum (Figure 1b).

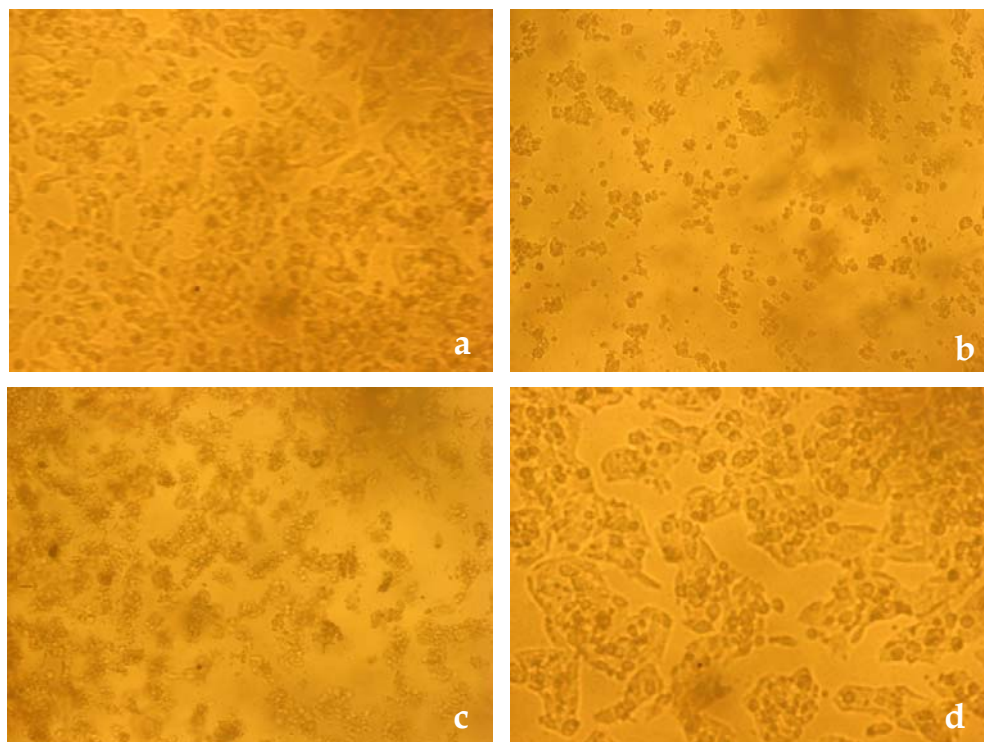


Figure 1. a. Normal MCF 7 cell lines; b. High toxicity (1000 $\mu$ g/ml); c. Medium Toxicity (250  $\mu$ g/ml); d. Low Toxicity (15.625 $\mu$ g/ml)

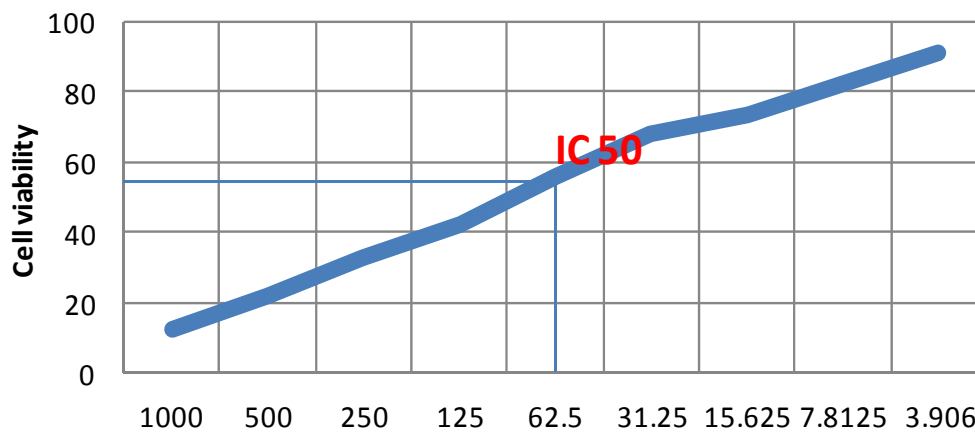


Figure 2. MTT assay

After treatment with 250µg of anthocyanin, the cells become spherical in shape and size with alter nuclear cytoplasm ratio (Figure 1c). This has indicated that anthocyanins render some changes on the cell surface associated with the adherence of the substratum (Kim *et al.*, 1999). Most of the cells had relatively flattened appearance with long multiple cytoplasmic processes forming cross bridges with neighbouring cells (Figure 1d).

#### **Inhibition of breast cancer line by anthocyanin:**

The anthocyanin extracts suppress the proliferation of MCF-7 cell lines (Figure 2). Cell viability decreased in a dose dependent manners. The result indicated that increasing concentration of anthocyanin from 3.9µg/ml to 1000µg/ml, the percentage of growth dilution of MCF cells increased progressively from 90.87% to 12.24%(Table 1).

Finally we reported that the anthocyanin extracted from *Musa acuminata* bract cause significant growth. Inhibition of human breast cancer cell line at 1000µg/ml concentration. Nam *et al.*, (2001) reported that polyphenols was able to inhibit tumor cells by apoptosis mediated cell inhibiton. The presence of a 4 carboxyl group of flavanoid molecule also contributes to anticancer activity (Plochman, 2007; Bravo 1998). Further, investigations into the identification of anthocyanin compounds present in the banana bract are needed to better elucidate their anticancer activities.

#### **Conclusion**

In the present study, anthocyanin extracted from *Musa acuminata* bract showed a strong anticancer activity against breast cancer cell line. This seems that the banana bract extract can be used as natural anticancer agent. Future investigation is being carried to identify and characteristic whether anthocyanin compounds responsible of anticancer activity of banana bract.

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#### **References**

- American Cancer Society (ACS). 2007. Cancer statistics 2007. Retrieved July 8, 2007. [http://www.cancer.org/docroot/stt/stt\\_0.asp](http://www.cancer.org/docroot/stt/stt_0.asp)
- Bai M, Agnantis NJ, Kamina S, Demos A, Zagorianakou P, Katsaraki A, Kanavaros P. 2001. In vivo cell kinetics in breast carcinogenesis. Breast Cancer Res 3:276-283.
- Bravo L, 1998. Polyphenols: chemistry, dietary sources, metabolism, and nutritional significance, Nutrition Reviews, vol. 56, no. 11, pp. 317-333
- Kim HS, Kacew S, Lee BY. 1999. In vitro chemopreventive effects of plantpolysaccharides (*Aloe barbadensis* Miller, *Lentinus edodes*, *Ganoderma lucidum* and *Coriolus versicolor*). Carcinogenesis 20:1637-1640.
- Kuo, P. L., Hsu, Y. L., Lin, T. C., Lin, L. T., and Lin, C. C. 2004. Induction of apoptosis in human breast adenocarcinoma MCF-7 cells by prodelphinidin B-2 3,3'-di-O-gallate from *Myrica rubra* via Fas-mediated pathway. J Pharm Pharmacol, 56: 1399-406.
- Miranda-Rottmann, S., Aspillaga, A. A., Perez, D. D., Vasquez, L., Martinez, A. L., and Leighton, F. 2002. Juice and phenolic fractions of the berry *Aristotelia chilensis* inhibit

LDL oxidation in vitro and protect human endothelial cells against oxidative stress. *J Agric Food Chem*, 50: 7542-7.

Mosmann, T.R., Bond, M.W., Coffman, R.L.,ohara, j. & Paul, W.E. 1983. T cell and mast cell lines respond to B cell stimulatory factor-1. *Proc. Natl Acad. Sci. USA*. 83, 5654.

Nam S, Smith DM and Dou QP: 2001. Tannic acid potently inhibits tumor cell proteasome activity, increases p27 and Bax expression, and induces G1 arrest and apoptosis. *Cancer Epidemiol Biomarkers Prev* 10: 1083-1088.

Plochmann K, Korte G, Koutsilieri E, 2007. Structureactivity relationships of flavonoid-induced cytotoxicity on human leukemia cells, *Archives of Biochemistry and Biophysics*, 460(1): 1-9.

Singletary KW, Stansbury MJ, Giusti M, Van Breemen RB, Wallig M, Rimando A. 2003. Inhibition of rat mammary tumnorigenesis by concord grape juice constituents. *J Agric Food Chem* 51:7780-7286.

Sporn MB, Suh N. 2000. Chemoprevention of cancer. *Carcinogenesis* 21:525-530.

Tapiero H, Tew K., Nguyen BaG, Mathé G. 2002. Polyphenols: do they play a role in the prevention of human pathologies? *Biomed Pharmacoter* 56: 200-207.

Tsuda, T., Horio, F., and Osawa, T. 2000. The role of anthocyanins as an antioxidant under oxidative stress in rats. *Biofactors*, 13: 133-9.

Yi, W., Fischer, J., Krewer, G., and Akoh, C. C. 2005. Phenolic compounds from blueberries can inhibit colon cancer cell proliferation and induce apoptosis. *J Agric Food Chem*, 53: 7320-9.

Young, G. P., and Le Leu, R. K. 2002. Preventing cancer: dietary lifestyle or clinical intervention? *Asia Pac J Clin Nutr*, 11 Suppl 3: S6 18-31.