Journal of
Plant Development Sciences
Meerut, India

RESEARCH ARTICLE

BAEL FRUIT DISPLAYED SIGNIFICANT POTENTIAL AGAINST LUNG & RENAL CANCER CELLS

Vikas Sharma[1]*, Navneet Kour[1], Komal Sudan[1], Shashank K Singh[2] and Shivangi Sharma[3]

[1]Natural Products Laboratory, Division of Biochemistry, Faculty of Basic Sciences, Sher-e-Kashmir University of Agricultural Sciences and Technology of Jammu, Main Campus Chatha, Jammu-180 009, J&K, India
[2]Cancer Pharmacology Division and Natural Product Chemistry Division, Indian Institute of Integrative Medicine, Canal Road Jammu-180001, J&K, India
[3]Department of Chemistry, Govt. Degree College, RS Pura -181102, Jammu, Jammu & Kashmir, India
Email: vikas.skuast@gmail.com

Received-28.04.2025, Revised-14.05.2025, Accepted-27.05.2025

Abstract: In the search of potential anticancer agents from minor fruits, the present research work was carried out to examine the *in vitro* cytotoxic potential of *Aegle marmelos* (bael) against nine human cancer cell lines from eight different origins such as MCF-7 and T-47D (breast), SF-295 (CNS), HCT-116 (colon), A-549 (lung), MDA-MB-435 (melanoma), OVCAR-5 (ovary), PC-3 (prostate), A-498 (renal). Methanolic extract of bael fruit was prepared and *in vitro* anticancer activity was determined *via* SRB assay at 100 µg/ml. Cells were allowed to grow for 24 h on 96-well flat bottom tissue culture plates and cells were further allowed to grow in the presence of test material for 48 h. Cell growth was terminated by addition of 50% (w/v) tricarboxylic acid and cells were stained with SRB dye. Excess dye was removed by washing with 1% (v/v) acetic acid and bound dye was dissolved in Tris buffer. OD was taken at 540 nm. *Aegle marmelos* displayed *in vitro* cytotoxic effect against colon, lung, melanoma and renal cancer cells and at lower concentrations of 50, 30, 10 and 1 µg/ml, the fruit exhibit significant *in vitro* cytotoxic effect against lung cancer cells (A-549). Further, IC_{50} value was calculated and it was observed that the bael fruit showed least IC_{50} value in case of lung cancer cells. To conclude, bael, possess certain constituents with cytotoxic properties and isolation / characterization of active ingredients is required for the development of anticancer drugs.

Keywords: *Aegle marmelos*, Bael, Cancer Cells, SRB assay, *In vitro* cytotoxicity

INTRODUCTION

Aegle marmelos L. Corr. has enormous traditional values against various diseases as fruit, leaves, stem, roots of the plant are used as ethno medicine against various human ailments (Badam *et al*., 2002 and Tomar 2012). Fruit part of bael possess high nutritional value and its pulp reported for the availability of steroids, terpenoids, flavonoids, phenolic compounds, lignin, fat / oil, inulin, proteins, carbohydrates, alkaloids, cardiac glycosides and flavonoids (Rajan *et al*., 2011). Methanol and aqueous extracts of bael fruit pulp were screened for antioxidant activity by DPPH radical scavenging method, reducing power assay, nitric oxide scavenging assay, superoxide radical scavenging assay, ABTS radical scavenging assay and H_2O_2 radical scavenging assay. Results demonstrated that both the extracts exhibited good antioxidant activity (Rajan *et al*., 2011). Antioxidant activity / free radical scavenging activity of the ripe and unripe fruit of *Aegle marmelos* was compared and it was found that the enzymatic antioxidants increased in ripe fruit when compared to unripe fruit extract (except glutathione peroxidase). The percentage of free radical inhibition was also high in unripe fruit than that of the ripe fruit (Sharmila and Devi, 2011). The anticancer effect of hydroalcoholic extract of bael was reported in the animal model of Ehrlich ascites carcinoma (Gagetia *et al*., 2005). In view of the above, *in vitro* anticancer potential of *A. marmelos*, has been investigated against nine human cancer cell lines from eight different tissues respectively.

MATERIALS AND METHODS

Fruit material and preparation of extract

Aegle marmelos, fruit part, was authenticated at site by Professor Vijay Bahadhur Singh and enough quantity of fresh fruits was collected from Rainfed Research Sub-station for Sub-tropical Fruits, Raya,

*Corresponding Author

SKUAST-Jammu. The freshly collected fruits were chopped, shade-dried and ground into powdered form. The methanolic extract of the fruit was prepared by percolating the dried ground plant material with 95% methanol and then concentrating it to dryness under reduced pressure to yield a crude extract (Kandil *et al.*, 1994). Stock solutions of 20 mg/ml were prepared by dissolving 95% methanolic extract in DMSO. Stock solutions were prepared at least one day in advance and were not filtered. The microbial contamination was controlled by addition of 1% gentamycin in complete growth medium *i.e.*, used for dilution of stock solutions to make working test solutions of 200 µg/ml.

***In vitro* assay for cytotoxic activity**

The fruit extract of bael was subjected to *in vitro* anticancer activity against various human cancer cell lines (Monks *et al.*, 1991). In brief, the cells were grown in tissue culture flasks in growth medium at 37 ^{o}C in an atmosphere of 5% CO_2 and 90% relative humidity in a CO_2 incubator (Hera Cell, Heraeus; Asheville, NCI, USA). The cells at sub-confluent stage were harvested from the flask by treatment with trypsin (0.05% trypsin in PBS containing 0.02% EDTA) and suspended in growth medium. Cells with more than 97% viability (trypan blue exclusion) were used for determination of cytotoxicity. An aliquot of 100 µl of cells (10^5 cells/ml) was transferred to a well of 96-well tissue culture plate. The cells were allowed to grow for 24 h. Extracts (100 µl/well) were then added to the wells and cells were further allowed to grow for another 48 h.

The anti-proliferative SRB assay which estimates cell number indirectly by staining total cellular protein with the dye SRB was performed to assess growth inhibition. The SRB staining method is simpler, faster and provides better linearity with cell number. It is less sensitive to environmental fluctuations and does not require a time sensitive measurement of initial reaction velocity (Skehan *et al.*, 1990). In brief, the cell growth was stopped by gently layering 50 µl of 50% (ice cold) trichloroacetic acid on the top of growth medium in all the wells. The plates were incubated at 4 ^{o}C for 1 h to fix the cells attached to the bottom of the wells. Liquid of all the wells was then gently pipetted out and discarded. The plates were washed five-times with distilled water and air-dried. SRB 100 µl (0.4% in 1% acetic acid) was added to each well and the plates were incubated at room temperature for 30 min. The unbound SRB was quickly removed by washing the cells five-times with 1% acetic acid. Plates were air-dried, tris buffer (100 µl, 0.01 M, pH 10.5) was added to all the wells to solubilize the dye and then plates were gently stirred for 5 min on a mechanical stirrer. The optical density (OD) was recorded on ELSIA reader at 540 nm. Suitable blanks (growth medium and DMSO) and positive controls (prepared in DMSO and distilled water) were also included. Each test was done in triplicate and the values reported were mean values of three experiments.

CALCULATIONS

The cell growth was determined by subtracting average absorbance value of respective blank from the average absorbance value of experimental set. Percent growth in presence of test material was calculated as under:

OD Change in Presence of Control = Mean OD of Control – Mean OD of Blank

OD Change in Presence of Test Sample = Mean OD of Test sample – Mean OD of Blank

% Growth in Presence of Control = 100/OD change in presence of control

% Growth in Presence of Test Sample = (% growth in presence of control) × OD change in presence of test sample

% Inhibition by Test Sample = 100 – % growth in presence of test sample

The growth inhibition of 70% or above was considered active while testing extracts

RESULTS AND DISCUSSION

The results revealed that the fruit extract from bael exhibits *in vitro* cytotoxic effect against four human cancer lines from four different origins at 100 µg/ml. The fruit displayed 79% growth inhibition of A-549 & A-498 cancer cells, 75% of MDA-MB-435 and 83% of HCT-116 cancer cells. When evaluated at lower concentrations, the fruit showed 74% growth inhibition at 50 µg/ml and 72% growth inhibition at 10 µg/ml against A-549 cancer cell line (Table 1). What is quite remarkable in these observations is that the cytotoxic effect shown by bael was much stronger than that shown by standard drugs for cancer (serving as positive controls in present investigation). Further, IC_{50} value was calculated and it was observed that bael fruit showed IC_{50}<10 µg/ml in case of lung and renal cancer cells (Fig. 1). The data was compared with literature values and it was found that the data was in good agreement with the published data. Extracts of bael were evaluated for anticancer potential using brine shrimp lethality assay, sea urchin eggs assay and MTT assay using tumor cell lines and the extracts exhibited toxicity on all used assays (Latica and Costa, 2005). Cancer is becoming a big load on families and economies. The cancer cases are on rise in Jammu and Kashmir with lung cancer becoming most prominent due to smoking. Cancer research has, therefore, become a major area of scientific research supporting the foundations of modern biology to a great extent. Chemotherapy is a major treatment modality for cancer, but most of the drugs used in cancer chemotherapy exhibit cell toxicity and can induce genotoxic, carcinogenic and teratogenic effects in non tumor cells. Therefore, the research for

alternative drugs of natural origin, which are less toxic, endowed with fewer side effects and more potent in their mechanism of action, is an important research line. Fruits have long history for the treatment of various diseases including cancer and active principles from these fruits are used to control the advance stages of malignancies in clinical settings. The results from the investigation forms a good basis for the selection of this minor fruit of Jammu region for further phytochemical and pharmacological analysis to offer new drugs from natural sources which would be less toxic and more potent for the efficient management of cancer. This promising methanolic fruit extract of bael can be explored for lead molecule (s) in the development of anticancer drugs to provide a great promise and service to lung cancer patients.

Table 1. Growth inhibitory effect of Bael fruit along with positive controls against human cancer cells

Generic name of the fruit	Extract	Conc. (µg/ml)	Human cancer cell lines from eight different tissues								
			Breast MCF-7	Breast T-47D	CNS SF-295	Colon HCT-116	Lung A-549	Melanoma MDA-MB-435	Ovarian OVCAR-5	Prostate PC-3	Renal A-498
			Growth Inhibition (%)								
Aegle marmelos	Methanolic	**100**	53	58	09	**83**	**79**	**75**	55	52	**79**
		50	*	*	*	51	**74**	00	*	*	57
		30	*	*	*	52	68	37	*	*	67
		10	*	*	*	00	**72**	09	*	*	59
		1	*	*	*	00	13	28	*	*	00
Positive control (standard drugs)		**Conc. (µM)**									
Doxorubicin		**1**	-	-	71	-	-	-	-	-	-
5-Fluorouracil		**20**	-	-	-	65	-	-	70	-	-
Mitomycin-C		**1**	-	-	-	-		-	-	63	-
Paclitaxel		**1**	77	72	-	-	71	-	-	-	70
Tamoxifen		**1**	-	-	-	-	-	75	-	-	-

Growth inhibition of 70% or more in case of extracts has been indicated in bold numbers
Mark (-) indicates that particular human cancer cell line was not treated with that particular positive control
Symbol (*) means not further evaluated

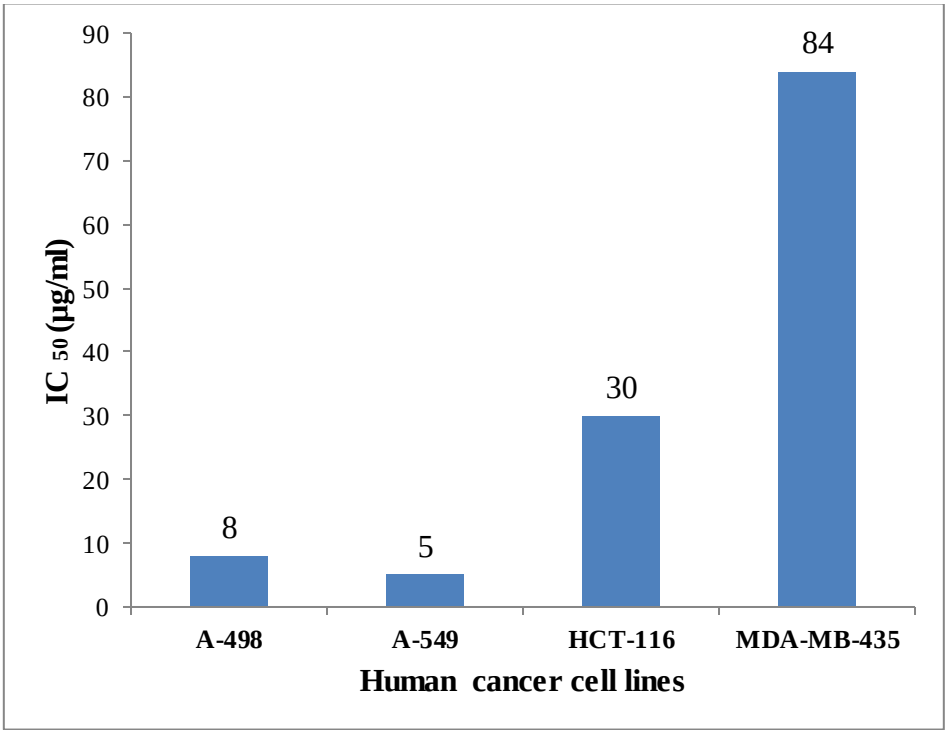

Fig. 1. IC_{50} value of methanolic extract of *Aegle marmelos* (bael)

ACKNOWLEDGEMENT

The authors are thankful to Rainfed Research Sub-station for Sub-tropical Fruits (RRSSF), Raya, SKUAST-Jammu for providing the raw/test material and CSIR-IIIM, Jammu for providing the technical support.

REFERENCES

Badam, L., Bedekar, S.S., Sonawane, K.B. and Joshi, S.P. (2002). *In vitro* antiviral activity of bael (*Aegle marmelos Corr*.) upon human Cox sack viruses B1-B6. *Journal of Communicable Diseases*, **34**: 88-95.

Google Scholar

Gagetia, G.C., Venkatesh, P. and Baliga, M.S. (2005). *Aegle marmelos* (L.) *Correa* inhibits the proliferation of transplanted ehrlich ascites carcinoma in mice. *Biological and Pharmaceutical Bulletin*, **28**: 58-64.

Google Scholar

Kandil, O., Radwan, N.M., Hassan, A.B., Amer, A.A.M., Banna, E.H.A. and Amer, W.M.M. (1994). Extracts and fractions of *Thymus capitatus* exhibit antimicrobial activities. *Journal of Ethnopharmacology*, **45**: 97-111.

Google Scholar

Monks, A., Scudiero, D., Skehan, P., Shoemaker, R., Paull, K., Vistica, D., Hose, C., Langley, J., Cronise, P., Vaigro-Wolff, A., Gray-Goodrich, M., Campbell, H., Mayo, J. and Boyd, M. (1991). Feasibility of a high-flux anticancer drug screen using a diverse panel of cultured human tumor cell lines. *Journal of National Cancer Institute*, **83**: 57-66.

Google Scholar

Skehan, P., Storeng, R., Scudiero, D., Monks, A., McMohan, J., Vistica, D., Warren, J. T., Bokesch, H., Kenney, S. and Boyd, M. R. (1990). New colorimetric cytotoxicity assay for anticancer-drug screening. *Journal of National Cancer Institute*, **82**: 1107-1112.

Google Scholar

Latica, V. and Costa, L. (2005). Evaluation of anticancer potential used in Bangladeshi folk medicine. *Journal of Ethnopharmacology*, **99**: 21-38.

Google Scholar

Rajan, S., Gokila, M., Jency, P., Brindha, P. and Sujatha, R.K. (2011). Antioxidant and phytochemical properties of *Aegle marmelos* fruit pulp. *International Journal of Current Pharmaceutical Research*, **3**: 65-70.

Google Scholar

Sharmila, S. and Devi, P.A.V. (2011). A review on pharmacological and phytochemical properties of *Aegle marmelos*. *Journal of Pharmacy Reasearch*, **4**: 720-722.

Google Scholar

Tomar, A. (2012). Medicinal uses of *Aegle marmelos* (Linn.) Corr. and *Bacopa monnieri* (Linn.) Pennel to cure Thyroid. *Journal of Non-Timber Forest Products*, 19(4): 301-302.

Google Scholar