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RESEARCH PAPER

Apricot kernel possesses *in vitro* cytotoxic effect against breast cancer cell lines

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Abstract : In the search for potential anticancer agents from fruits, the present research work was carried out to examine the *in vitro* cytotoxic potential of kernel part of *Prunus armeniaca* (apricot) against eight distinct human cancer cell lines from six different tissues: lung (A-549), colon (HCT-116), breast (MCF-7, MDAMB-231), pancreatic (MIAPaCa-2, Panc-1), prostrate (PC-3) and CNS (N2A). Methanolic extract and subsequent fractions (n-hexane, chloroform, ethyl acetate, acetone and methanol) were used as test material and anticancer activity was determined *via* SRB assay at 100µg/mL. Results revealed that chloroform fraction of kernel suppressed the proliferation of human breast cancer cellline with growth inhibition of 89%. The fraction of kernel was then evaluated at lower concentrations of 50, 40, 30, 20 and 10µg/mL. Further IC_{50} value was calculated and it was observed that the fraction showed IC_{50}38.66. To conclude, kernel part of *Prunus armeniaca* possesses certain constituents with cytotoxic properties that can be used to develop anticancer agents especially for breast cancer therapy and to provide a great service to cancer patients.Further studies are required for the isolation of active ingredients from the kernel part.

Key Words : *Prunus armeniaca* (apricot), Kernel, SRB assay, Breast cancer

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INTRODUCTION

Cancer is perhaps the most perilous infections, which addresses a considerable burden in the society and appears being an excellent reason for concern. Multidisciplinary scientific examinations are putting forth best attempts to battle the disease, yet the definite shot, perfect cure is yet to be brought into the universe of medication. Natural products devoured in our day by day diet could be an answer for this lethal illness by giving chemoprotective and chemotherapeutic cure. Natural products display a wide scope of antimicrobial, anticarcinogenic andantiproliferation activities. These biological activities can be ascribed to their antioxidant properties (Ren *et al*., 2003). They contain a huge assortment of substances having antioxidant activity such as vitamin C, vitamin E, carotenes, polyphenols and flavonoids (Chanwitheesuk *et al*., 2005; Campbell *et al*., 2013; Dragovic *et al*., 2005; Dulf *et al*., 2017). The apricot (*Prunus armeniaca*) is an individual from the

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Rosaceae family and comprises of around 175 species appropriated everywhere. Apricot trees are not universal since they can just fill in specific districts where the ecological conditions are good (Bolarinwa *et al.,* 2014). Apricot ought to be viewed as a good source of natural antioxidant for foods and functional food source against malignancy and heart disease (Yigit *et al*., 2009). Apricot fruit contains sugars, vitamin C and K, niacin, thiamine, minerals, β-carotene, organic acids, phenols, flavonoids, volatile compounds, esters, dietary proteinand significant amounts of oil and fibers (Haciseferoullari *et al*., 2007). Hence, the target of the current investigation was to determine the antitumor exercises of methanolicextract and subsequent fractions (n-hexane, chloroform, ethyl acetate,acetone and methanol) of apricot kernels.

MATERIAL AND METHODS

Chemicals likeDMEM medium, RPMI-1640 medium, dimethyl sulfoxide (DMSO), EDTA, fetal bovine serum (FBS), sulphorhodamine blue (SRB) dye, phosphate buffer saline (PBS), trypsin, gentamycin, penicillin and 5-flurouracil were purchased from Sigma Chemical Co., USA. All other chemicals were of high purity and purchased locally from Ramesh Traders, Panjthirthi-Jammu, J&K, under the brands Sigma-Aldrich Chemicals Pvt. Ltd. and S.D. Fine Chemicals Pvt. Ltd.

Preparation of extracts and fractions:

A sufficient amount of fresh fruits of apricot were collected from the Kargil region of Union Territory of Ladakh. The newly gathered fruits were cut and the kernels were taken out, shade-dried and crushed into a powdered form. The dried powdered plant material was percolated with 95 per cent methanol and then concentrated to dryness under decreased pressure to make the methanolic extracts of the kernel. Further fractionation of the crude (methanolic) extract was performed using n-hexane, chloroform, ethyl acetate and acetone. All fractions were concentrated and kept at-20^{0}C till further investigation. 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 100 μg/mL.

Cell lines/cultures and positive controls:

The human cancer cells lung (A-549), colon (HCT-116), breast (MCF-7 and MDAMB-231), pancreatic (MIAPaCa-2 and Panc-1), prostrate (PC-3) and CNS (N2A) were obtained from National Centre for Cell Science, Pune, India and National Cancer Institute, Frederick, USA. These human cancer cells were further grown and maintained in RPMI-1640 and DMEM medium. Doxorubicin, 5-Fluorouracil, Mitomycin-C and Paclitaxel were used as positive controls.

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

Extract and subsequent fractions were subjected to *in vitro* anticancer activity against different human cancer cell lines(Monks *et al.,* 1991). In brief, the cells were grown in tissue culture flasks in growth medium at 37^{0}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 subconfluent stage were gathered from the flask by treatment with trypsin (0.05% trypsin in PBS containing 0.02% EDTA) and suspended in growth medium. Cells with over 97% viability were utilized for determination of cytotoxicity. An aliquot of 100iL of cells was moved to a 96-well tissue culture plate. The cells were permitted to develop for 24 hrs. Extract and fractions (100 μL/well) were then added to the wells and cells were additionally allowed to grow for another 48 hrs. To assess growth inhibition, the anti-proliferative SRB test was used, which measures cell number indirectly by staining total cellular protein with the dye. SRB staining is easier, quicker and offers greater linearity with cell number. It is less affected by environmental changes and does not need a time-sensitive assessment of initial reaction velocity(Skehan*et al.,* 1990).

In brief, cell growth was inhibited by carefully stacking 50 L of 50% (ice cold) trichloroacetic acid on top of the growth medium in all wells. The plates were incubated at 40°C for 1 hour to adhere the cells to the bottom of the wells. The liquid from each well was then carefully pipetted out and discarded. The plates were washed five-times with distilled water and air-dried. SRB 100μL (0.4 % in 1% acetic acid) was applied to each well and the plates were incubated for 30 minutes at room temperature. By washing the cells five times with 1% acetic acid, the unbound SRB was promptly eliminated. Plates were air-dried before adding tris buffer (100μL, 0.01 M, pH 10.5) to all wells to solubilize the

dye. Plates were then gently swirled for 5 minutes on a mechanical stirrer.At 540 nm, the optical density (OD) was measured using an ELSIA reader. Positive controls (made in DMSO and distilled water) and appropriate blanks (growth medium and DMSO) were also included. Each test was performed in triplicate and the results given were the mean of three trials.

Cell growth was calculated by subtracting the average absorbance of the corresponding blank from the average absorbance of the experimental set. The percentage increase in the presence of test substance was computed as follows:

– 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

When evaluating extracts and fractions, a growth inhibition of 70% or greater was deemed active, however, while testing active components at varying molar concentrations, a growth inhibition of 50% or greater was considered active.

RESULTS AND DISCUSSION

The SRB test was used to examine the cytotoxic impact of extracts and fractions of apricot kernel. The

Table 1 : Growth inhibitory effect of kernel part of *Prunus armeniaca* against eight different human cancer cell lines

Generic name of the fruit	Conc. (µg/ml)	Human cancer cell lines from six different tissues							
Prunus armeniaca		Breast		Colon	CNS	Lung	Pancreatic		Prostate
(kernel)		MCF-7	MDAMB-231	HCT-116	N2A	A-549	MIAPACA	Panc-1	PC-3
Extract					Growth inhibition (%)				
(Methanolic)	100	64	51	00	08	30	50	10	12
Fraction (Hexane)	100	47	29	00	00	20	19	00	02
Chloroform	100	89	54	00	20	27	64	18	14
Ethyl acetate	100	29	14	00	00	13	30	00	0
Acetone	100	37	26	00	07	44	29	17	01
Methanol	100	00	01	00	00	19	15	00	01
Positive controls (standard drugs)	Conc. (µM)								
Doxorubicin	1	65	65	-	71	-	-	-	-
5-Fluorouracil	20	-	-	52	-	-	-	-	-
Mitomycin-C	1	-	-	-	-	66	-	-	66
Paclitaxel	1	-	-	-	-	-	87	87	-

Mark (-) indicates that particular human cancer cell line was not treated with that particular positive control

Table 2 : Growth inhibitory effect of chloroform fraction prepared from kernel part of *Prunus armeniaca* against breast cancer cell line (MCF-7)

Fraction	Conc. (µg/ml)	Human cancer cell line from breast origin
		Breast (MCF-7)
		Growth inhibition (%)
Chloroform	100	89
	50	82
	40	50
	30	22
	20	7
	10	0
IC_{50} (µg/ml)		38.66
Positive controls (standard drugs)	Conc.(µM)	
Doxorubicin	1	65
Paclitaxel	1	78

kernel'smethanolic extract at 100µg/mL, seems to be inactive against eight human cancer cell lines from six different tissues namely lung (A-549), colon (HCT-116), breast (MCF-7 and MDAMB-231), pancreatic (MIAPa Ca-2 and Panc-1), prostrate (PC-3) and CNS (N2A) as it supressed 64 %, the highest inhibition against breast cancer cell line (MCF-7), 51% against MDAMB-231, 50 % against MIAPaCa-2, 30 % against A-549, 12 % against PC-3, 10 % against Panc-1, 8 % against N2A and 0 % against HCT-116 along with positive controls as shown in Table 1. The fractions namely n-hexane, ethyl acetate, acetoneand methanol did not exhibit any cytotoxic effect against any of the eight human cancer cell lines. However, chloroform fraction showed significant growth inhibition of 89% against only MCF-7, a cancer cell line from breast origin. The chloroform fraction was subjected to lower concentration (50, 40, 30, 20 and 10µg/mL) in order to calculate its IC_{50}. At lower concentrations, chloroform fraction of kernel showed 82% growth inhibition at50µg/mL, 50% growth inhibition at 40µg/mL, 22% growth inhibition at 30µg/mL, 7% growth inhibition at 20µg/mL and 0% growth inhibition at 10µg/mL against breast cancer cell line (MCF-7). Further, IC_{50} value was calculated and it was observed that kernel showed IC_{50} 38.66µg/mL in case of breast cancer cell line (MCF-7) as depicted in Table 2.

Cancer is putting a significant strain on families and economies. Increased fruit eating has been proven in epidemiological research to avert a substantial number of cancer-related deaths. High fruit consumption protects against many cancers and the preventive benefits of high fruit consumption are ascribed to vitamins/minerals and phytochemicals, which have the ability to modulate human metabolism in a way that is beneficial for cancer prevention.As a result, fruits taken as part of our regular diet may be a cancer-fighting strategy and the synergistic effects of phytochemicals in fruits are responsible for their powerful antioxidant/anticancer properties (Erdogan and Kartal, 2011; Fan *et al*., 2018; Garcia *et al*., 2016; Hussain *et al*., 2013). The current study was designed to assess the *in vitro* anticancer potential of *Prunus armeniaca* kernel (collected from the Kargil region of Union Territory of Ladakh) against eight human cancer cell lines from six distinct tissues. Our *in vitro* cytotoxic experiment employing SRB dye and conducted with suitable standard drugs for cancer(positive controls) showed interesting results from medicinal view point as the chloroform fraction of kernel part of apricot exhibited significant *in vitro* cytotoxic activity against human breast cancer cell line. When the data was compared to published numbers, it was discovered that the data agreed with the published data.Human liver carcinoma (HepG2) and human breast cancer (MCF-7) were studied using extracts of *Prunus armeniaca* kernel and it was found that human liver cancer cells and human breast cancer cells were suppressed by methanol, ethanol and water extracts of kernel part of *Prunus armeniaca* (Gomaa, 2013; Chen *et al*., 2020).

To conclude, *Prunus armeniaca* kernel part showed significant efficacy against breast cancer cells (MCF-7) at lower doses, with an IC_{50} of 38.66µg/mL. The current study attempted to determine the *in vitro* anticancer potential of *Prunus armeniaca* kernel collected from the Kargil region of Union Territory of Ladakh. Our findings validated the therapeutic potential of *Prunus armeniaca*, particularly against breast cancer cells. The findings also revealed that the fruit has cytotoxic components that might be utilized to produce anticancer medicines for breast cancer treatment. Furthermore, it provides a solid foundation for the selection of the fruit's kernel for further phytochemical and pharmacological study and it provides us with novel medicines from natural sources that are less toxic and more potent for the effective management of cancer.

REFERENCES

Bolarinwa, I.F., Orfila, C. and Morgan, M.R. (2014). Amygdalin content of seeds, kernels and food products commercially-available in the UK. *Food Chemistry*, **152**: 133-139.

Campbell, O.E., Merwin, I.A. and Zakour, O.I. (2013). Characterization and the effect of maturity at harvest on the phenolic and carotenoid content of northeast USA apricot (*Prunus armeniaca*) varieties. *Journal of Agricultural and Food Chemistry*, **61**(51): 12700-12710.

Chanwitheesuk, A., Teerawutgulrag, A. and Rakariyatham, N. (2005). Screening of antioxidant activity and antioxidant compounds of some edible plants of Thailand. *Food Chemistry*, **92**: 491-497.

Chen, Y., Ghamdi, A.A., Elshikh, M.S., Shah, M.H., Al-Dosary, M.A. and Abbasi, A.M. (2020). Phytochemical profiling, antioxidant and Hep G2 cancer cells antiproliferation potential in the kernels of apricot cultivars. *Saudi Journal of Biological Sciences*, **27**(1): 163-172.

Dragovic, V., Delonga, K., Levaj, B., Djakovic, S. and Pospisil, J. (2005). Phenolic profiles of raw apricots, pumpkins and their purees in the evaluation of apricot nectar and jam

authenticity. *Journal of Agricultural and Food Chemistry*, **53**(12): 4836-4842.

Dulf, F.V., Vodnar, D.C., Dulf, E.H. and Pintea, A. (2017). Phenolic compounds, flavonoids, lipids and antioxidant potential of apricot (*Prunus armeniaca* L.) pomace fermented by two filamentous fungal strains in solid state system. *Chemistry Central Journal*, **11**(1): 1-10.

Erdogan, I. and Kartal, M. (2011). Insights into research on phytochemistry and biological activities of *Prunus armeniaca* L. (apricot).*Food Research International*, **44**(5): 1238-1243.

Fan, X., Jiao, W., Wang, X., Cao, J. and Jiang, W. (2018). Polyphenol composition and antioxidant capacity in pulp and peel of apricot fruits of various varieties and maturity stages at harvest. *International Journal of Food Science and Technology*, **53**(2): 327-336.

Garcia, M.C., González-Garcia, E., Vasquez-Villanueva, R. and Marina, M.L. (2016). Apricot and other seed stones: Amygdalin content and the potential to obtain antioxidant, angiotensin I converting enzyme inhibitor and hypocholesterolemic peptides. *Food and Function*, **7**(11): 4693-4701.

Gomaa, E.Z. (2013). *In vitro* antioxidant, antimicrobial, and antitumor activities of bitter almond and sweet apricot *(Prunus armeniaca* L.) kernels.*Food Science and Biotechnology*, **22**(2): 455-463.

Haciseferooullari, H., Gezer, I., Ozcan, M.M. and Asma, B.M. (2007). Postharvest chemical and physical mechanical properties of some apricot varieties cultivated in Turkey. *Journal of Food Engineering*,**78**: 364-373.

Hussain, P.R., Chatterjee, S., Variyar, P.S., Sharma, A., Dar, M.A. and Wani, A.M. (2013). Bioactive compounds and antioxidant activity of gamma irradiated sun dried apricots (*Prunus armeniaca* L.). *Journal of Food Composition and Analysis*, **30**(2): 59-66.

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**: 757-766

Ren, W., Qiao, Z., Wang, H., Zhu, L. and Zhang, L. (2003). Flavonoids: promising anticancer agents. *Medicinal Research Reviews,* **23**: 519-534.

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

Yigit, D., Yigit, N. and Mavi A. (2009). Antioxidant and antimicrobial activities of bitter and sweet apricot (*Prunus armeniaca* L.) kernels. *Brazilian Journal of Medical and Biological Research*, **42**: 346-352.