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**Anti-cancer activity of *Morus nigra*
leaves extract**

Anti-cancer activity of *Morus nigra* leaves extract

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Abstract

Plants are screened for treatment of many ailments including cancer because they possess certain potential constituents which are effective for treatment. The aim of this study was to evaluate the anti-cancer activity of *Morus nigra* leaves against human cervical cancer cell line (HeLa). *n*-Hexane and aqueous methanolic extract of plant's leaves were made by maceration. Anti-cancer activity was estimated by methyl-thiazolyl-tetrazolium (MTT) assay and percentage inhibition of cells was calculated. Results of MTT showed that 100 µg/mL aqueous methanol extract of *M. nigra* inhibited 89.5 - 32.0% of HeLa cell line. It was concluded that *M. nigra* possess anti-cancer activity.

Introduction

Cancer is one of the leading death causing disease of the current era and is characterized by aberrant growth of cell mass that is uncoordinated to remain proliferating when stimulus has been removed that provoked that action. Many plants have recently be reported with anticancer activity like *Casuarina equisetifolia* (Shafiq et al., 2014), *Aspergillus niger* (Channabasavaet al., 2014) and *Convolvulus arvensis* (Saleem et al., 2014).

M. nigra (Family Moraceae), commonly as Black Mulberry (English) and Shah-toot (Hindi/Urdu), is used as hepatoprotective (Malhi et al., 2014), anti-oxidant (Imran et al., 2010; Ercisli and Orhan, 2008) and antimicrobial (Digrak et al., 1999).

The objective of this study was to evaluate the anti-cancer activity of *M. nigra* extracts against human cervical cancer cell line ((HeLa).

Material and Method

Collection of medicinal plants

M. nigra leaves weres collected from Jinnah colony, plant nurseries located at Bilal road and Samundri road, Faisalabad. The plant material was identified from

Department of Botany, University of Agriculture, Faisalabad. Prior to maceration, all plants were washed, shadow dried and grinded to coarse powder for extract formation.

Preparation of extracts

One thousand three hundred grams of powdered *M. nigra* (leaves) was merged in 4,000 mL of *n*-hexane and aqueous methanol (70% methanol and 30% distilled water) for 7 days with occasional shaking. After maceration, rotary evaporator lyophilizer was used for concentration of the extracts.

Anti-cancer activity

Single cell suspension was made by trypsin-ethylene-diamine tetraacetic acid (EDTA) and layer of cells was separated. Final density of 1×10^5 was made using medium containing 5% FBS and diluted the cell suspension. In 96-well plates, 10,000 cells/well were seeded and incubated at 37°C, keeping 5% CO₂, 95% air and 100% relative humidity. After 24 hours, different concentrations of extracts i.e., 1, 10, 25, 50 and 100 µg/mL were added and incubated under above mentioned working conditions for another 48 hours. Well without plant extracts were taken as control. Whole procedure was repeated in triplicate and viable cells were calculated using hemocytometer before and after



extracts addition (Dantuet al., 2012).

MTT assay

In every well 100 μ L of MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) in phosphate buffered saline was added and incubated at 37°C for 4 hours. The medium with MTT was flipped off and the formed formazan crystals was solubilized in 100 μ L of DMSO. Using micro plate reader the absorbance was measured at 490 nm. The %cell inhibition was determined using the following formula (Cheng et al., 2011).

$$\% \text{Cell inhibition} = 1 - \frac{\text{Absorbance sample} \times 100}{\text{Absorbance control}}$$

Graph was plotted against concentrations to calculate IC₅₀.

Statistical analysis

A logistic linear regression model was fit to the data using Microsoft Excel 2013 Software to calculate the IC₅₀. The data obtained were expressed as mean \pm standard deviation. A value of p<0.05 was considered as significant.

Results and Discussion

The anti-cancer activity of *n*-hexane and aqueous methanolic extract of *M. nigra* (leaves) against HeLa cancer cell line is shown in Table I. 100 μ g/mL aqueous methanol extract of *M. nigra* inhibited 89.5-32.0% of HeLa cell line. Estimated IC₅₀ of *n*-hexane and aqueous methanolic of *M. nigra* against HeLa cancer cell line at 24 hours was 185.9 \pm 8.3 and 56.0 \pm 1.7 μ g/mL respectively. *n*-Hexane and aqueous methanolic extract at 1, 10, 25, 50 and 100 μ g/mL had shown dose dependent inhibition of cells.

Sundararajan et al. (2006) also evaluated anti-cancer activity of *n*-hexane extract of *Bidens pilosa* on HeLa and KB cell lines corroborated our findings with IC₅₀ values of 509.2 \pm 6.3 and 385.2 \pm 4.7 μ g/mL respectively. A lower IC₅₀ of *Carthamus oxyacanthus* (whole plant)

| Table I | | |
|--|------------------|------------------|
| Percentage inhibition (Mean \pm SD) of HeLa cell line by <i>Morus nigra</i> extracts | | |
| Concentration (μ g/mL) | <i>n</i> -Hexane | Aqueous methanol |
| 1 | 0.04 \pm 0.0 | 0.8 \pm 0.1 |
| 10 | 4.5 \pm 0.8 | 7.2 \pm 1.1 |
| 25 | 17.9 \pm 0.5 | 20.8 \pm 2.2 |
| 50 | 30.9 \pm 0.1 | 48.1 \pm 1.3 |
| 100 | 41.2 \pm 0.8 | 89.5 \pm 2.0 |
| IC ₅₀ | 185.9 \pm 8.3 | 56.0 \pm 1.6 |

indicates that it has phytochemical constituents that synergistically inhibit growth of cancer cells (Alesiani et al., 2010).

It can be concluded that *M. nigra* (leaves) possess anti-cancer activity against cervical (HeLa) cancer cell line.

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