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Anti-cancer activity of *Ruellia squarrosa* against human prostate cancer cell line

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Abstract

Ruellia species are traditionally used for the treatment of flu, fever and inflammation. The aim of this study was to evaluate the anti-cancer activity of *Ruellia squarrosa* extracts against human prostate cancer (PC3) cell line. Dichloromethane and methanol extracts of aerial and root parts of the plant were made by maceration. Anti-cancer activity was estimated by MTT assay and percentage inhibition of cells was calculated. Results of MTT showed that 30 mg/ml of dichloromethane extract of root parts of the plant showed moderate anti-cancer activity (58% inhibition) against PC3 cell line with IC_{50} 15.4 ± 0.3. It was concluded that *R. squarrosa* (root) possesses anti-cancer activity against human prostate cancer (PC3) cell line.

Introduction

Ongoing research is being focused to seek out effective treatments for cancer. It is hoped that search for compounds having significant anti-cancer and cytotoxic activities in plants may contribute to finding effective anti-cancer therapeutics. Many plants have recently reported with anti-cancer activity like *Aspergillus niger* (Channabasava et al., 2014) and *Morus nigra* (Qadir et al., 2014).

Ruellia squarrosa belonging to family Acanthaceaeis commonly known as "Water Bluebell" or "Neeli Daisy". *Ruellia* species are traditionally used for the treatment of flu, fever and inflammation and scientifically have been proved to be antipyretic, analgesic, and antioxidant (Chen et al. 2006).

The objective of this study was to evaluate the anti-cancer activity of *R. squarrosa* extracts against human prostate cancer (PC3) cell line.

Materials and Methods

Preparation of extracts

The aerial and root parts of *R. squarrosa* were collected from the surrounding of BZU, Multan. The plant was identified by Prof. Altaf Ahmed Dasti, Institute of Pure and Applied Biology, BZU, Multan. Voucher specimens No. Fl.C. 1-2 were deposited in the herbarium. The shade dried plant material (roots and aerial parts) of *R. squarrosa* were ground; then subjected for maceration with dichloromethane and methanol for three days successively. The both dichloromethane and methanol extracts were concentrated by using the rotary evaporator.

Anti-cancer assay (MTT assay)

Anti-cancer activity was recorded in 96-well microplates by MTT assay. Human prostate cancer cells (PC3) were cultured in DMEM (Dulbecco's Modified Eagle's Medium), along with 5% of FBS (fetal bovine serum), 100 IU/mL of penicillin and 100 µg/mL of streptomycin in 75 cm^2 flasks, and kept in 5% CO_2 incubator at 37°C. Exponentially growing cells were harvested, counted with hemocytometer and diluted with a particular medium. Cell culture with the concentration of 1 x 10^5 cells/mL was prepared and introduced (100 µL/well) into 96-well plates. After incubation, medium was removed and 200 µL of fresh medium was added with concentrations of compounds

(1-30 µM). After 48 hours, 200 µL MTT (0.5 mg/mL) was added to each well and incubated further for 4 hours. 100 µL of DMSO was added to each well. The extent of MTT reduction was calculated by measuring the absorbance at 570 nm, using a micro plate reader. The cytotoxicity was measured as concentration causing 50% growth inhibition (IC_{50}) for PC3 cells. The percent inhibition was determined by using the following formula.:

$$\%\ Cell\ Inhibition = [1 - \frac{Absorbance\ Sample}{Absorbance\ Control} \times 100]$$

Graph was plotted against concentrations to calculate IC_{50}.

Statistical analysis

A logistic linear regression model was fit to the data using Microsoft Excel 2013 to calculate the IC_{50}. The data obtained were expressed as mean ± SD. A value of $p<0.05$ was considered as significant.

Results and Discussion

Dichloromethane and methanol extracts of roots and aerial parts of *R. squarrosa* were evaluated for anti-cancer activity by MTT assay against human prostate cancer (PC3) cell line. The anti-cancer activity of extracts is given in Figure 1. The dichloromethane extract of root part of the plant showed moderate anti-cancer activity against PC3 cells line with IC_{50} 15.4 ± 0.3 using doxorubicin as standard.

In the previous studies, extracts of *Convolvulus arvensis* have shown cytotoxic effects against Colo 205 cells (Kaur and Kalia, 2012), *Morus nigra* against human cervical cancer cell line (HeLa) (Qadir et al., 2014), *Bidens pilosa* against HeLa and KB cell lines (Sundararajan et al. 2006) and *Carthamus oxyacanthus* against murine B16-F1 melanoma cells (Alesiani et al., 2010). We have evaluated anti-cancer activity of *R. squarrosa* against human prostate cancer cell lines. MTS analysis indicated the antiproliferative activity of methanol and dicholoromethane extracts of Aerial parts as well as roots of the plant. For all the extracts, decline in cell proliferation was dose dependent. At a dose of 30 mg/ml, there was a sharp decline in cell proliferation by dicholoromethane extract of roots of *R. squarrosa*. At this dose almost 42% of the total cells survived and 58% became dead.

Ruellia species are rich in flavonoids (Lin et al. 2006). The anti-cancer activity of flavonoids has already been established (Kandaswami et al., 2005).

Conclusion

The anti-cancer activity of *R. squarrosa* may be due to the flavonoids present in it.

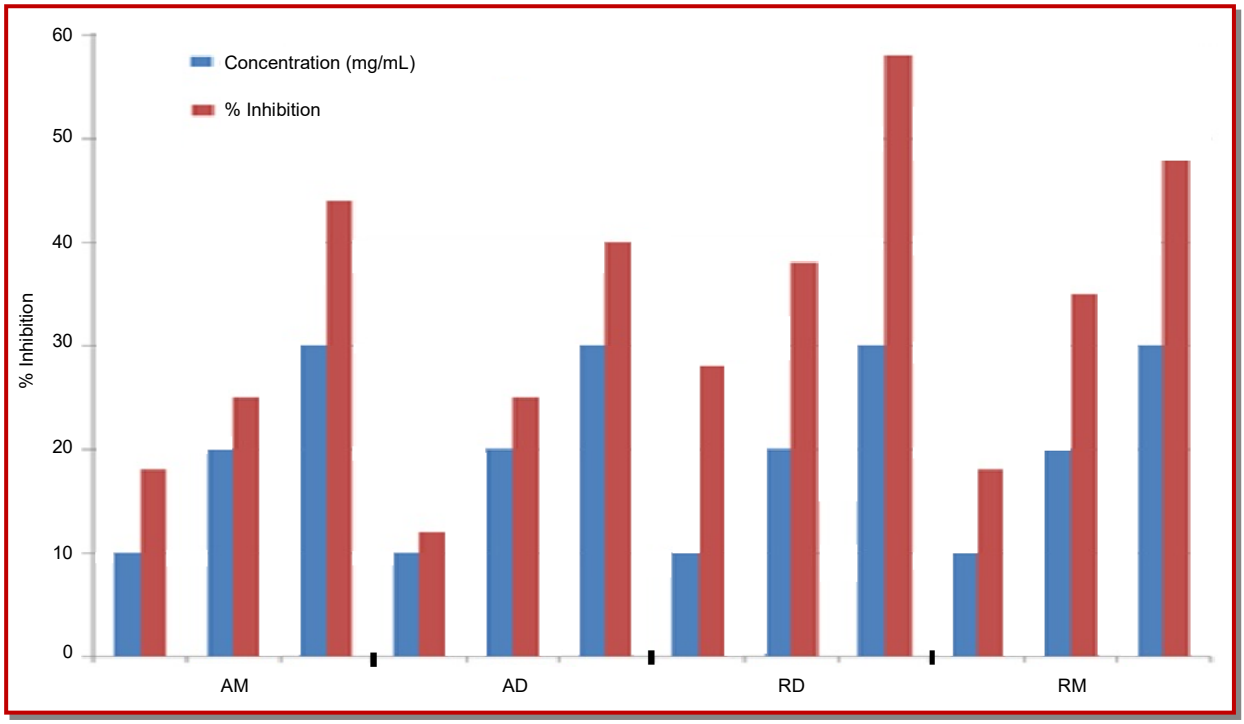

Figure 1: Anti-cancer activity of *R. squarrosa* (A: Aerial parts; R: Roots; M: Methanol extract; D: Dicholoromethane extract)

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Conflict of Interest

Authors declare no conflict of interest

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