RESEARCH ARTICLE

Cytotoxicity Assessment of Six Different Extracts of *Abelia triflora* leaves on A-549 Human Lung Adenocarcinoma Cells

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Abstract

The present investigation was designed to assess the anticancer activity of six different leaf extracts (ethyl acetate, methanol, chloroform, petroleum ether, n-butanol, and water soluble) of *Abelia triflora* on A-549 human lung adenocarcinoma epithelial cells. A-549 cells were exposed to 10-1000 µg/ml concentrations of the leaf extracts of *A. triflorafor* 24 h and then percentage cell viability was assessed by 3-(4,5-dimethylthiazol-2yl)-2,5-biphenyl tetrazolium bromide (MTT) assay. The results showed that leaf extracts of *A. triflora* significantly reduced the viability of A-549 cells in a concentration-dependent manner. Decrease was recorded as 31% with ethyl acetate, 36% with methanol, 46% with chloroform, 54% with petroleum ether, 62% with n-butanol, and 63% with water soluble extracts at 1000 µg/ml each. Among the various plant extracts, ethyl acetate extract showed the highest decrease in the percentage cell viability, followed by methanol, chloroform, petroleum ether, n-butanol, and water soluble extracts. Our results demonstrated preliminary screening of anticancer activity of different soluble extracts of *A. triflora* extracts against A-549 cells, which can be further used for the development of a potential therapeutic anticancer agents.

Keywords: *Abelia triflora* - A-549 cell line - cytotoxicity - MTT Assay

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Introduction

Cancers figure among the leading causes of morbidity and mortality worldwide, with approximately 14 million new cases and 8.2 million cancer related deaths in 2012 (WCR, 2014).The deaths from cancer are projected to continuously rise worldwide, with an estimated 13.1 million deaths in 2013 (Orang-Ojong et al., 2013). Lung cancer is the second most common cancer in men, the colon and rectum cancers in combination rank third in frequency among males in the United States.

Anticancer drugs are used to target all rapidly proliferating cancer cells. Chemotherapy is one of the most important therapeutic options used to treat human cancers, either alone or in combination with radiation therapy and surgery (Al-Oqail et al., 2013; Powathil et al., 2014). Although a number of new anti-cancer drugs that target various key processes in cancer progression have been developed, but overall patient survival rates have mostly remained unchanged for a number of years. Hence, the use of natural products has now been looked upon thoughtfully in the control of cancer. Plant derived natural products have received considerable attention in recent years due to the presence of various bioactive compounds and their diverse pharmacological properties including cytotoxic and cancer preventive effects (Ong et al., 1986; Owen et al., 2004; Omar, 2010; Patel et al., 2010; Karmakar et al., 2010; Al-Oqail et al., 2013; Farshori et al., 2013; Al-Sheddi et al., 2014; Farshori et al., 2014). The genus *Abelia* (Caprifoliaceae) consists of about eighty species distributed mostly in Himalaya and East Asia.A.triflorahas a wide distribution throughout from China to the Himalayas. *Abelia triflora* R. Br. is the only species found in Pakistan. In Pakistan it is found in Kaghan Valley (Hazara District) between 1500-3000 m, on dry rocky ground (Perveen and Qaiser, 2007). Available literature reveals that only little work has so far been done on A. triflora. Reports showed that only a few irridoid and bisirridoid glycosides have been isolated from *Abelia grandiflora* and *Abelia chinensis* (Murai et al., 1985; Tomassini et al., 2000) of genus Abelia. In one of the study, the methanol extract of *A. triflora* plant showed strong toxicity in brine shrimp lethality test.Furtherfractionationsof methanolic extract revealed strong toxicity in ethyl acetate and n-butanol soluble sub-fractions (Olowa and Nuneza, 2013). The non-

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availability of anticancer activity of this plant prompted us to carry out this study to screen the anticancer activities of different leaf extracts of *A. triflora* against human lung adenocarcinoma epithelial cell line (A-549).

Materials and Methods

Chemicals and consumables

Solvents and all other specified reagents were purchased from Sigma Chemical Company Pvt. Ltd. St. Louis, MO, USA. DMEM culture medium, antibiotics-atimycotic solution, fetal bovine serum (FBS), and trypsin were purchased from Invitogen, Life Sciences, USA. Consumables and culture wares used in the study were procured from Nunc, Denmark.

Cell Line and cell culture

Human lung adenocarcinoma epithelial cell line(A-549) was cultured in DMEM, supplemented with 10% FBS, 0.2% sodium bicarbonate, and 1% antibiotic/antimycotic solution. Cells were grown in 5% CO_2 at 37°C in high humid atmosphere. Before the experiments, viability of cells was assessed following the protocol of (Siddiqui et al., 2008). A-549 cells showing more than 98% cell viability and passage number between 10 and 12 were used in this study.

Plant material and extraction

Leaves (8.0 kg) of A. trifloraplantswere collected in July 2012, from Ziarat Valley near Quetta, Baluchistan province, Pakistan and identified by plant taxonomist of the Department of Botany, Baluchistan University, Quetta, where a voucher specimen (#300) has been deposited in the herbarium of that department.The shade dried leaves (8.0 kg) were ground and extracted with methanol (3×10L) at room temperature. The combined methanol extract (M) was evaporated under reduced pressure to obtain a thick gummy mass. It was suspended in water and successively extracted with petroleum ether (P), chloroform (C), ethyl acetate (E), n-butanol (B), and water soluble fraction (W).

Experimental design

A-549 cells were exposed to various concentrations of different soluble extracts of Abeliatriflora(10 µg/ml to 1000 µg/ml) for a period of 24 h. Following the exposures, cells were subjected to assess the cytotoxic responses by 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide, MTT assay.

Drug solutions

The extracts were not completely soluble in aqueous medium; therefore the stock solutions of all the extracts were prepared in dimethylsulphoxide (DMSO) and diluted in culture medium to reach the desired concentrations. The concentration of DMSO in culture medium was not more that 0.1% and this medium was used as control.

Cytotoxicity assessments by MTT assay

Percentage cell viability was assessed using the 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide (MTT) assay using the describedprotocol (Siddiqui et al., 2008). Briefly, A-549 cells ($1x10^4$) were seeded in 96 well culture plates and were allowed to adhere for 24 h CO_2 incubator at 37°C. After 24 h exposure, MTT (5 mg/ml of stock in PBS) was added (10 µl/well in 100 µl of cell suspension), and plates were incubated further for 4 h. Then, supernatants were discarded and 200 µl of DMSO were added to each well and mixed gently. The developed color was read at 550 nm using multiwell microplate reader (Thermo Scientific, USA). Untreated sets were also run under identical conditions and served as control.

Statistical analysis

The results were expressed as mean and standard error of means (SEM). One way ANOVA was employed to detect differences between the groups of treated and control. The values showing $p<0.05$ were considered as statistically significant.

Results

Cytotoxicity assessment of plant extracts

The *in vitro* cytotoxic effect of different soluble extracts of Abeliatriflorawasassessedby the MTT assay.

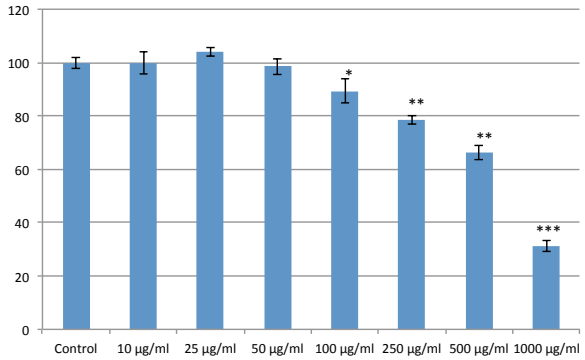

Figure 1. Cytotoxicity Assessments by MTT Assay in A-549 Cells Following the Exposure of Various Concentrations of Ethyl Acetate Extract of Abelia Triflora Leaves for 24 h. Values are mean±SE of three independent experiments. *p<0.05, **p<0.01, ***p<0.001 *Vs* Control

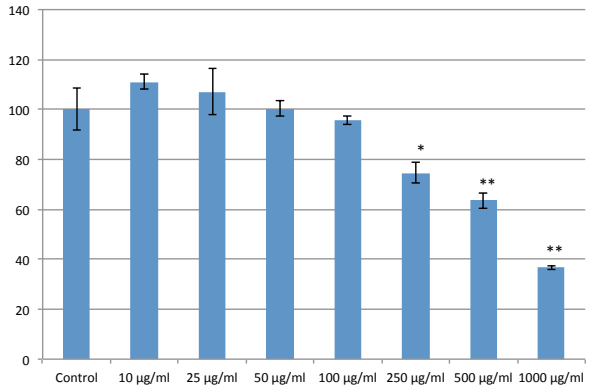

Figure 2. Cytotoxicity Assessments by MTT Assay in A-549 Cells Following the Exposure of Various Concentrations of Methanol Extract of Abelia Triflora Leaves for 24 h. Values are mean±SE of three independent experiments. *p<0.01, **p<0.001 *Vs* Control

The results in percentage cell viability of different extracts are presented in Figures 1-6. The results showed that A. trifloraleaf extracts induced cytotoxicity in human lung adenocarcinoma epithelial cell line(A-549). It was found that the tested extracts significantly reduced cell viability in a concentration-dependent manner. Among the various plant extracts, ethyl acetate soluble extract (E) showed the highest decrease in the percentage cell viability, followed by methanol (M), chloroform (C), petroleum ether (P), n-butanol (B), and water soluble (W) extracts. The ethyl acetate solubleextract has shown decrease in the cell viability of A-549 cells at 100 µg/ml and above concentrations exposed for 24 h. The cell viability of A-549 cells at 100, 250, 500, and 1000 µg/ml of ethyl acetate extract were recorded as 89%, 78%, 66%, and 31% respectively (Figure 1). Whereas, the methanol, chloroform, petroleum ether, butanol, and water soluble plant extracts have shown decrease in cell viability at 250 µg/ml and above concentrations. The cell viability of A-549 cells were recorded as 74%, 63%, and 36% in methanol extract (Figure 2), 87%, 76%, and 46% in chloroform extract (Figure 3), 91%, 80%, and 54% in petroleum ether extract (Figure 4), 89%, 73%, and 62% inn-butanol extract (Figure 5), and 90%, 86%, and 63% in water extract (Figure 6) at 250, 500, and 1000 µg/ml, respectively. The methanol, chloroform, petroleum ether, n-butanol, and water soluble extracts of *Abelia triflora* at100 µg/ml and lower concentrations did not show any decrease in the percentage cell viability of A-549 cells except ethyl acetate solubleextract (Figures 1-6).

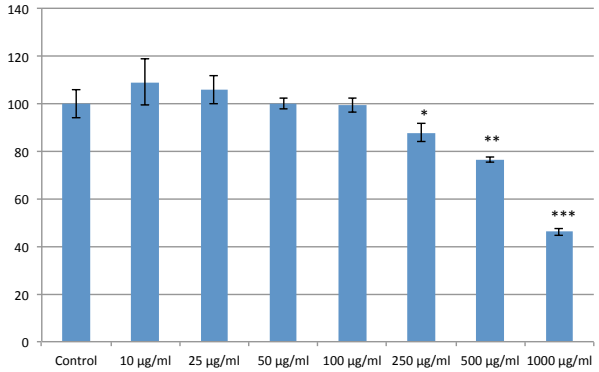

Figure 3. Cytotoxicity Assessments by MTT Assay in A-549 Cells Following the Exposure of Various Concentrations of Chloroform Extract of Abelia Triflora Leaves for 24 h. Values are mean±SE of three independent experiments. *p<0.01, **p<0.001, ***p<0.001 *Vs* Control

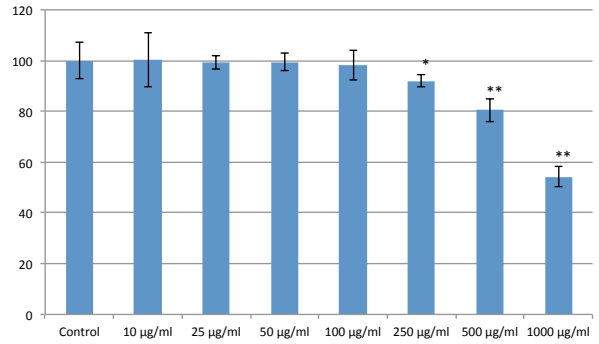

Figure 4. Cytotoxicity Assessments by MTT Assay in A-549 Cells Following the Exposure of Various Concentrations of Petroleum Ether Extract of Abelia Triflora Leaves for 24 h. Values are mean±SE of three independent experiments. *p<0.05, **p<0.001 *Vs* Control

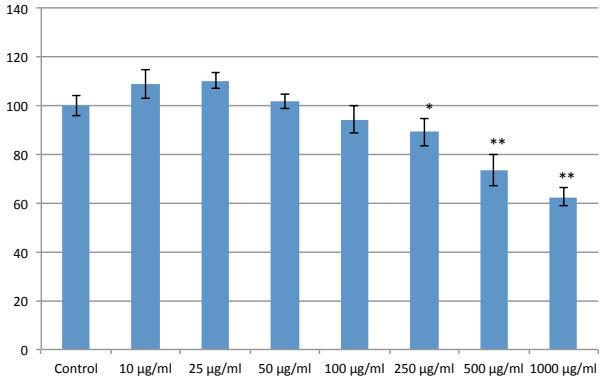

Figure 5. Cytotoxicity assessments by MTT assay in A-549 cells following the exposure of various concentrations of n-butanol extract of Abelia triflora leaves for 24 h. Values are mean±SE of three independent experiments. *p<0.01, **p<0.001, ***p<0.001 *Vs* Control

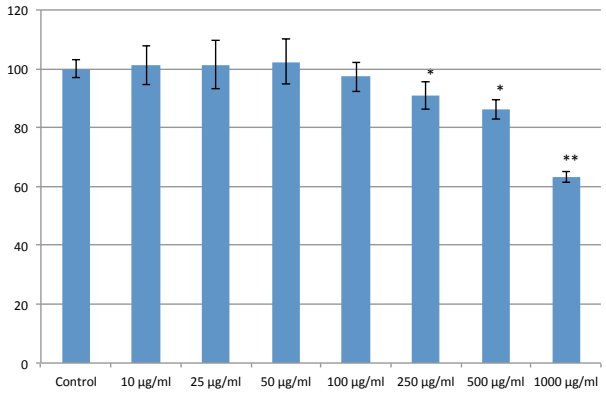

Figure 6. Cytotoxicity Assessments by MTT Assay in A-549 Cells Following the Exposure of Various Concentrations of water Extract of Abelia Triflora Leaves for 24 h. Values are mean±SE of three independent experiments. *p<0.05, **p<0.001 *Vs* Control

Discussion

The belief that natural medicines are much safer than synthetic drugs has led to a resurgence of scientific interest in their biological effects. The second-hand metabolites produced in medicinal plants have many applications in the treatment of various human diseases. Recent reports have cited that plants and their components could act as tumor suppressor and apoptotic inducers in cancerous cells (Al-Oqailet al., 2013; Farshori et al., 2013; Al-Sheddiet al., 2014; Farshori et al., 2014). The genus *Abelia* has not been explored much and only a few irridoid and bisirridoid glycosides have been isolated from *Abelia grandiflora* and *Abelia chinensis* (Murai et al., 1985; Tomassini et al., 2000). However, no evidence of pharmacological work on this genus was found, thus, the present investigation was carried out to screen the anticancer activities of

different leaf extracts of *A. triflora* against human lung adenocarcinoma epithelial cell line (A-549).Our results demonstrate the *in vitro* cytotoxic effect of different soluble extracts of Abeliatrifloraassess by the MTT assay. The results showed that A. trifloraleaf extracts significantly reduced the cell viability ofhuman lung adenocarcinoma cell line (A-549) in a concentration-dependent manner. Among the various plant extracts, ethyl acetate soluble extract was found more toxic than the other soluble extracts i.e. methanol, chloroform, petroleum ether, n-butanol, and water solubleextracts. The ethyl acetate solubleextract has shown decrease in the cell viability of A-549 cells at 100 µg/ml and above concentrations exposed for 24 h. However, the methanol, chloroform, petroleum ether, butanol, and water soluble plant extracts have shown decrease in cell viability at 250 µg/ml and above concentrations. There has been no published report on the effect of Abeliatriflora extract on the human lung adenocarcinoma cell line so far, but the growth inhibitory effects of other extracts on cancerous cells have been observed by different investigators on other human cancer cell lines (Li et al., 1995; Kim et al., 2002; Kumi-Diaka and Butler, 2000). Our results are in well agreements with the previous studies where the exposures of various natural products have been shown to induced significant cytotoxic effects against different cancer cell lines (Srividya et al., 2012; Al-Oqail et al., 2013, Farshori et al., 2013; Al-Sheddi et al., 2014; Farshori et al., 2014). Our results also correlate with previous findings showing the *in vitro* cytotoxicity in this concentration range (Abdullah et al., 2014). Other plant extracts have also been shown to induce cytotoxicity against human lung adenocarcinoma cell line, A-549 (Solowey et al., 2014; Manglani et al., 2014). It has also been concluded that this kind of effects towards cancerous cells may be due to the presence of bioactive components such as, polysaccharides, flavonoids, coumarins, monoterpene glycoside and alkaloids in these plant extracts (Xiang et al., 2005, Xin et al., 2008, Li et al., 2009, Tan et. al., 2013).

Our results demonstrated, for the first time, that *Abelia triflora* extracts significantly reduced the cell viability of human lung cancer cells (A-549) in a concentration-dependent manner *in vitro*. Our findings also support therapeutic use of *Abelia triflora* extracts as an anticancer agent which will be useful to integrate for improving modern cancer care. Further experimental analyses are required, to obtain more detail mechanism (s) of action for the development of new drug in the treatment of cancer. This study will also be useful to the researchers working in this area to take forward the references for further evaluation of anticancer activity.

Acknowledgements

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