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Research Article

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The methanolic and aqueous extracts of *Viburnum lantana* exhibit antitumor activity *in vitro*

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ABSTRACT

Several plant-derived drugs have been used in medical oncology till now. In this study the methanolic and aqueous extracts of Viburnum lantana were tested for possible antitumor activity using potato disc assay. To investigate the antimicrobial activity the minimum inhibitory concentration (MIC) of 0.01, 0.1 and 1 mg/ml of each extract against Agrobacterium tumefaciens was evaluated using microplate method. The antitumor activity of the extracts was evaluated with the potato disc assay. Both aqueous and methanolic extracts demonstrated no antibacterial activity. In potato disc assay a dose response correlation was observed between the extracts and the tumor growth inhibition. In conclusion, V. lantana methanolic and aqueous extracts did not exert inhibitory effects on bacterial growth, however, inhibition effect on the tumor growth observed in a dose-dependent manner. These results confirm the anti-tumor activity of V. lantana.

Key words: *Viburnum lantana*, *Agrobacterium tumefaciens*, Potato disc assay, Cytotoxic activity, Tumor inhibition.

INTRODUCTION

Most of current cancer chemotherapies have severe side effects on normal mammalian cells.[1] They cause risks of nephrotoxicity, neurotoxicity, vascular toxicity, infertility, thromboembolic complications, hair loss, nausea, myocardial infarction.[2]

Due to the various side effects and inadequacy of the common treatments, many researches are devoted to replace cancer therapy drugs to new agents more specific against uncontrolled proliferative cells.

To date, some chemoprevention studies have been successfully designed, the studied agents tested and clinically used. For example, dactinomycin and doxorubicin are microorganisms based compounds. Herbal agents have also been used for chemoprevention such as vinblastine, irinotecan, topotecan, vincristine and taxanes.

In this regard, different plant species are tested to find proper cytotoxicity. *Viburnum* is one of such plants, a genus from the Caprifliaceae family which is known in medicinal herbs and comprises more than 230 species. Various therapeutic uses of *Viburnum* in different areas of the world are reported; such as diuretic, astringent, antispasmodic, treatment of tumefaction and diarrhea anti-asthma anti diabetic and cytotoxic.[3-7]

Viburnum lantana, a species of *Viburnum*, is a dense shrub with grooved leaves and small black fruits.[8]

In this research, we tested the cytotoxic ability of aqueous and methanolic extracts of *V. lantana* on a potato disk tumor growth inhibition model.

EXPERIMENTAL SECTION

Plant material:
V. lantana was collected from Chamestan forest, Noor city, Golestan province, Iran during spring season. The collected plants were botanically identified and a voucher specimen (No. 189-1-12) was deposited in herbarium of School of Pharmacy, Mashhad University of Medical Sciences, Iran. The plant leaves were separated and dried in shadow, and then grounded to fine particles of 1 mm mesh sizes.

Microorganism and Culture media:
Agrobacterium tumefaciens (strain B6) cells containing Ti (tumor inducing) plasmid were cultured on Petri dishes containing Soybean Casein Digest Agar (SCDA) medium. Agar slants were incubated at 25°C for 24 hours to 10 days.

For antitumor tests bacterial cells were inoculated into Tryptone Soya Broth (Soybean Casein Digest Medium) and incubated at 25˚Cuntil the optical density at 560 nm was 0.4 (about 1.0×10^8 ml^{-1} CFU).

Preparation of methanolic and aqueous extract:
The dried and powdered leaves were successively extracted in a Soxhlet apparatus with 250 ml of absolute methanol for 12 hours, at a temperature lower than the solvent boiling point. Crude aqueous extract was obtained using a maceration method adding 400ml of cold distilled water to 100g of powdered plant material and incubated at 40°C for 24 h in a shaking flask.

Finally, all extracts and solutions weresterilized using a 0.22 µm filter.
Antibacterial activity of the extracts:
Serial dilutions of 0.01, 0.1 and 1 mg/ml of both aqueous and methanolic extracts were prepared at a ratio of 6:4 were diluted with Soybean Casein Digest Broth (SCBD) medium. Serially diluted extracts were mixed with 1 ml of *A. tumefaciens* suspension (10^8 CFU/mL). 1ml of each suspension was added to a microplate well and incubated at 25°C for 16 h. To determine bacterial growth, 0.4 ml of triphenyltetrazolium chloride (5mg/ml) added to each well and incubated at 25°C. After 30 minutes the optical densities of the wells were read out in a microplate reader (545 nm). Minimum inhibitory concentration (MIC) of each dilution was calculated as (the absorption of tetrazolium added bacterial suspensions -absorption of tetrazolium solution).Gentamicin was used as positive control.

Cytotoxicity assay:
The antitumor activity of the extracts was evaluated with the potato disc method. Moderate sizes of white potato (*Solanum tuberosum* L.) tubers were submersed for 30 minutes in 1% w.v solution of sodium hypochlorite. Disinfected pieces were washed by sterilized water and cut to discs with 15 mm in diameter and 5 mm in height, then placed in 1.5 % agar containing sterile Petri dishes.

To inoculate the discs, different dilutions of methanolic and aqueous extracts in water were mixed with a 10^8 CFU/ml suspension of *A. tumefaciens* in separated tubes. Sterilized vincristine sulfate and water were used as positive and negative controls, respectively.

50 µl of each tube was added on potato discs and the dishes were incubated under a laminar air cabinet and at 25^0C for 21 days. 21 days later, the disks were stained with Lugol's reagent to count the number of tumors. At last, the percentage of tumor inhibition was determined.

Statistical analysis:
The data were analyzed using an analysis of variance (ANOVA) model and presented as mean ± SD followed by Tukey-kramer. Statistical analysis was performed using Instant software and significance was defined as $p< 0.05$.

RESULTS AND DISCUSSION:

In the cancer-fighting field, cancer prevention, is more important for researchers, than cancer treatment (9), hence, food and medicinal plants anticancer agents containing are important facets of cancer prevention in biomedical research (10). The aim of the study was to evaluate antiproliferative properties of methanolic and aqueous extracts of *V. lantana*, considering their growth-inhibitory effect on *A. tumefaciens*.

A. tumefaciens is a gram-negative bacterium, the causative agent of crown gall, a neoplastic disease in plants which occurs in more than 60 families of dicotyledon. Potato disc bioassay is the base of evaluating new antitumor agents in animals, for their effect on the initiation of crown gall tumor on potato. Tumor blocking may happen for two reasons: antibacterial activity (inhibition of *A. tumefaciens* growth) or anti-tumor activity.

In the present study, *in vitro* antibacterial activity of the aqueous and methanolic extracts was studied against *A. tumefaciens*. Both aqueous and methanolic extracts at 0.01-1 mg/ml doses showed no inhibitory effect on bacterial growth (data not shown).

The inhibitory effect of *V. lantana* methanolic and aqueous extracts against the crown gall tumour growth was compared in different dilutions using potato disc bioassay. As the positive control, tumor formation on potato disc was completely blocked by 0.08 mg/ml of vincristine sulfate (100% inhibition), (Figure 1, 2).

The tumor growth inhibition percentage at any dilution was calculated by 1-n, where the "n" parameter refers to the ratio at equilibrium of tumor numbers of each dilution to the negative control × 100. The Values are the mean ± S.D. (Tukey–Kramer, $P < 0.001$).

In a dose dependent manner, in comparison with the negative control, all doses of both aqueous and methanolic extracts of *V. lantana* showed antitumor activity. The percentage of blocking was more than 20% ($P<0.001$). The IC_{50} values were calculated to be 0.05 and 1.13 mg/ml for the aqueous and methanolic extracts respectively.

These results confirm the anti-tumor activity of *V. lantana*. The aqueous extract was approximately more potent than the methanolic extract in the inhibition of crown galltumour growth. This observation is in agreement with a previous report that aqueous extract of *V. lantana* contains anti-oxidant compounds (11). It is interesting to note that Altun et al. in an investigation of water extract of *Viburnum lantana* leaves showed a slight anti-inflammatory and a powerful antinociceptive activity (12).Additional studies are required for determining *V. lantana*'s compounds, especially concerning the anti-tumor aqueous extract ingredients.

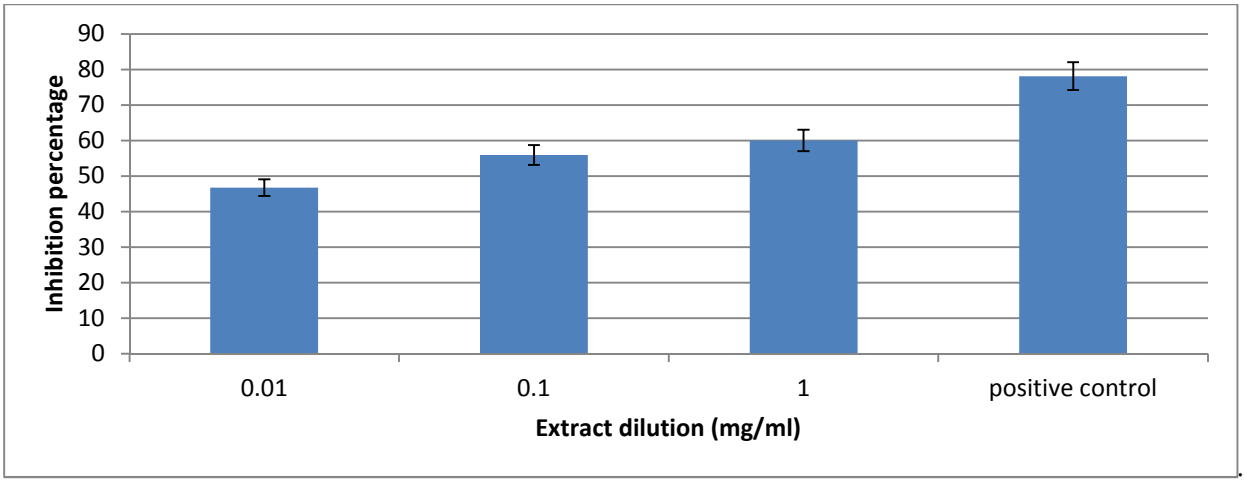

Figure 1: Crown gall tumour growth inhibition of aqueous extract of *V. lantana*

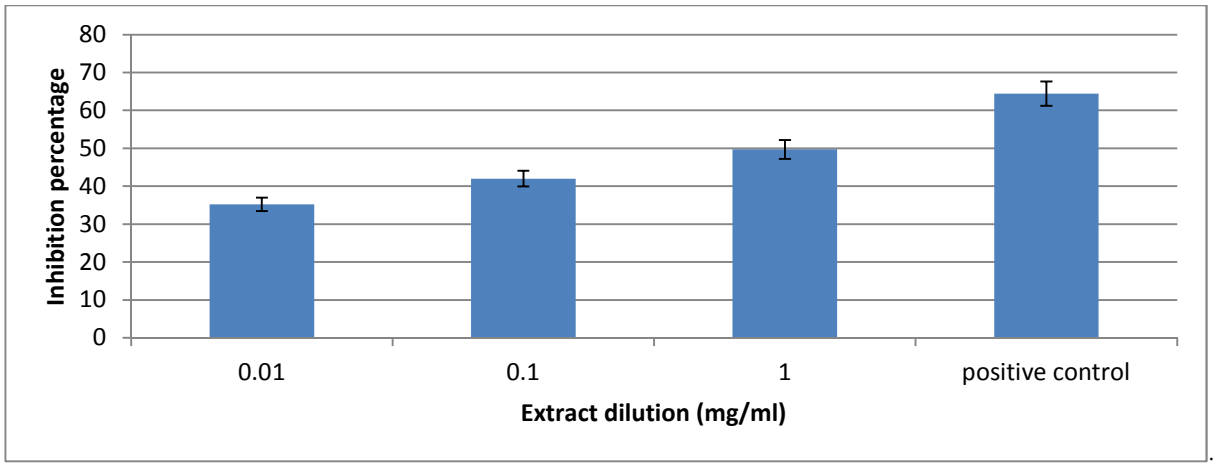

Figure 2: Crown gall tumour growth inhibition of methanolic extract of *V. lantana*

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