

The Anticancer Potential of *Pavonia odorata* Willd. Extract on Human Breast (MD-MB-231), Prostate (PC-3) and Lung (Calu-6) Cancer cell lines

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ABSTRACT

Introduction: A huge reservoir of bioactive compounds exists in many species of plants, only a small percentage of which have been examined and continued to be an important source of anticancer agents. Worldwide effects are ongoing to identify new anticancer compounds from plants. With the current decline in the number of new molecular entities from the pharmaceutical industry, novel anticancer agents are being sought from traditional medicines. **Objective:** In the present study we investigated the efficacy of methanol extracts of *Pavonia odorata*, for its clonogenic inhibition on Human Breast cancer (MD-MB-231), Prostate cancer (PC-3) and Lung cancer (Calu-6) cell lines. **Materials and Methods:** The cytotoxic effect was evaluated by MTT assay. **Results:** The methanol extract of *P. odorata* showed significant cytotoxicity against MD-MB-231 and Calu-6, when compared to PC-3 cells. **Conclusion:** The methanol extracts of *P. odorata*, showed effective cytotoxic activities in a dose dependent

manner. Future work will be interesting to know the chemical composition and also better understanding the mechanism of action will help in developing it as drug for therapeutic application.

Key words: Alternative medicine, Anti-cancer, Drugs, Indian Medicinal plants, Inhibition.

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DOI : 10.5530/pj.2016.1.6

INTRODUCTION

Herbal drugs have been used since ancient times as medicines for the treatment of a range of diseases. Medicinal plants have played a key role in world health. An increasing number of research papers and reviews clearly indicate that medicinal plants exhibit a variety of therapeutic properties¹⁻³ and provide health security to the people. *In vitro* cytotoxicity screening models provide important preliminary data to help select plant extracts with potential antineoplastic properties for further work to develop them into therapeutic leads. As mentioned above, the exploration of nature as a source of new active agents is needed for discovering bioactive chemo-types from natural product for the development and novel molecular diversity of efficacious drugs. In this respect, natural products from plants, used either alone or with combinatorial synthetic methodologies, constitute a multidisciplinary approach to the current drug productivity.^{4,5} This has opened up new fields of investigation of potential antitumor compounds, some of which are already widely used in cancer chemotherapy which act through different pathways in activation of apoptosis in cancer cells leading to cell cytotoxicity.

Pavonia odorata (Malvaceae) is an indigenous medicinal plant of India that is frequently used in Ayurvedic system of medicine as a cooling, diaphoretic, diuretic, and demulcent.^{6,7} In combination with others, it is prescribed as an astringent and tonic for fever, inflammation and haemorrhage.^{7,8} It has been reported for anti-inflammatory and anti-microbial activities.⁹ In an earlier study it was shown that the hydro alcoholic fractions of *P. odorata* showed *in vitro* cytotoxicity against Erlich's ascites carcinoma (EAC) cells.¹⁰ Further, it was observed that the hydroalcoholic fractions significantly inhibited the ascites tumor in EAC bearing mice.¹⁰ As till date, there was no report on its anticancer potential of *Pavonia odorata* (*P. odorata*) on other cancer cell lines, henceforth, in this study we have attempted to investigate the *in vitro* cytotoxic poten-

tial of methanolic extract of *P. odorata* on Human Breast cancer (MD-MB-231), Prostate cancer (PC-3) and Lung cancer (Calu-6) cell lines.

MATERIALS AND METHODS

Materials

All the chemical reagents and solvents of analytical grade were purchased from SRL Chemicals, India. 3-(4, 5- dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide (MTT, No M5655) purchased from Sigma (St Louis, MO, USA).

PLANT MATERIAL AND EXTRACTS PREPARATION

Selection of Medicinal plants

Pavonia odorata Willd. (*P. odorata*) were collected from Western Ghats and around Mysore. Plant were analyzed for botanical identity, scientific name including genus, species, subspecies or variety and the family of the plant were recorded and verified for taxonomical status, as recommended by the national pharmacopoeia. Further, the plants were identified by a Taxonomist and the voucher specimens (SY-P-52) were kept at G7 Synergon Private Limited.

Preparation of extracts

P. odorata plant parts were washed with distilled water, dried and crushed into fine powder by using electric grinder. The finely powdered material was extracted with methanol in a Soxhlet apparatus for 24 h. The extract was dehydrated/concentrated under reduced pressure using a rotary flash evaporator (BuchiFlawil, Switzerland) and preserved aseptically at 5°C in airtight bottle until further use.¹¹

In-vitro cytotoxic assays

Cell culture assay

The human carcinoma cell lines, MDA-MB-231, PC 3 and Calu 6 were obtained from American Type culture Collection (ATCC). The cells were grown in DMEM containing 2 mM L-glutamine supplemented with 10% fetal bovine serum and 100 U/ml of penicillin-streptomycin. The cells were incubated at 37°C in a humidified 5% CO₂ incubator. All the cell lines used in this study were of early passage number.

MTT Assay

Cytotoxicity assay

Cytotoxicity test were carried out using MTT assay.^{12,13} The Trypsinize 70-80% confluent cell lines (MDA-MB-231, PC 3 and Calu 6) of 1×10⁵ cells/well are seeded in a 96 well plate and incubate for 24 hr at 37°C, and varying concentrations (0-500 µg/ml) of *P. odorata* were added. After incubation of different time points, 20 µL of MTT reagent were added to each well and incubated for 4 hr at 37°C. The incubated cells were washed twice with PBS and DMSO (100 µL/well) reagent which dissolved the insoluble crystalline formazan product. The efficacy of the sample was determined based on the reduced dye at 570 nm by UV spectrophotometer. Doxorubicin at 50 µm/ml was used as a standard and appropriate controls were taken. The effects of the samples on the proliferation of cell lines were expressed as the % cell viability, using the following formula:

% cell viability = A_{570} of treated cells / A_{570} of control cells × 100%.

Statistical analysis

The experiments were carried out in triplicate and results are given as the mean ± standard deviation. The data in all the experiments were analyzed (Microsoft Excel 2007) for statistical significance using Students *t*-test and differences were considered significant at *p*<0.05.

RESULTS AND DISCUSSION

Plants have always been a potential source of new drug molecule and research work in this domain has resulted in discovery of more efficient drugs for cancer treatment.^{3,5} MTT is a simple, reliable technique, which measures cell viability and can be used for screening anti-proliferative agents.^{12,13} MTT assay is a spectrophotometric analysis, which uses (3-[4,5-dimethyl-thiazol-2-yl]-2,5-diphenyl tetrazolium bromide), known as MTT, a yellow color and water soluble compound. The MTT enters the cells through the plasma membrane and, in contact with superoxide produced by the mitochondrial activity, is oxidized to MTT-formazan, a slat purplish color, which is insoluble in water. Then the oxidation of MTT is proportional to the mitochondrial activity and therefore to cell viability.^{12,13}

In the present study, we investigated the efficacy of methanol extracts of *P. odorata*, for its clonogenic inhibition on Human Breast cancer (MD-MB-231), Prostate cancer (PC-3) and Lung cancer (Calu-6). When evaluated at different concentration (0-500 µg/ml) it was observed that in a dose dependent manner the extract of *P. odorata* was effective in inducing cytotoxic effect upon MD-MB-231 (Figure 1), PC-3 (Figure 2) and Calu-6 (Figure 3) cell lines. When compared, the methanol extract of *P. odorata* exhibited significant cytotoxicity up to ~80%. However, doxorubicin which acted as positive control exhibited up to ~86%. Based on the IC₅₀ values (Table 1), it is observed that the methanol extract *P. odorata* showed significant cytotoxicity against MD-MB-231 and Calu-6, when compared to PC-3 cells. Furthermore, it was observed that the control assays that were carried out for samples containing only the appropriate volumes of blank solutions showed no effect on cell growth. In an earlier study it was observed that cytotoxic and antitumor effect was observed for chloroform, ethyl acetate and hydroalcoholic fraction of *P. odorata* against EAC cells.¹⁰ Further, it was observed that in the

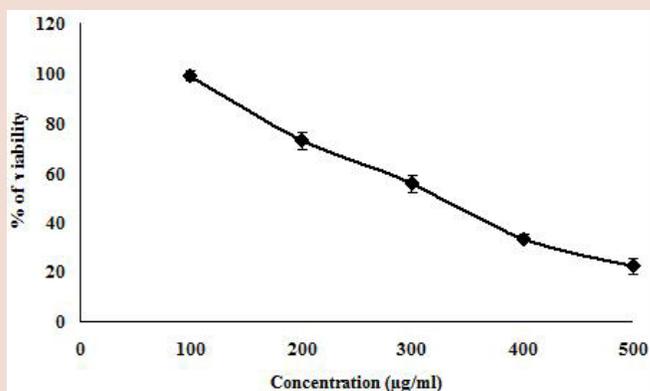


Figure 1: Cytotoxic activity of *P. odorata* methanolic extract on Human Breast cancer (MD-MB-231) cell lines.

Extract were incubated with 10⁵ viable cells at concentrations ranging from 0 to 500 µg/ml for 48 h. Cell viability was determined by the MTT method.

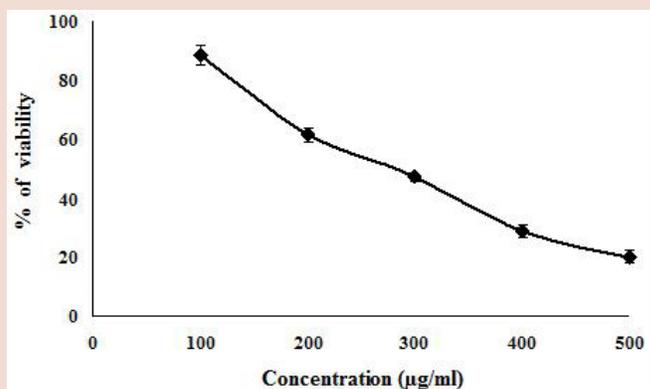


Figure 2: Cytotoxic activity of *P. odorata* methanolic extract on Prostate cancer (PC-3) cell lines.

Extract were incubated with 10⁵ viable cells at concentrations ranging from 0 to 500 µg/ml for 48 h. Cell viability was determined by the MTT method.

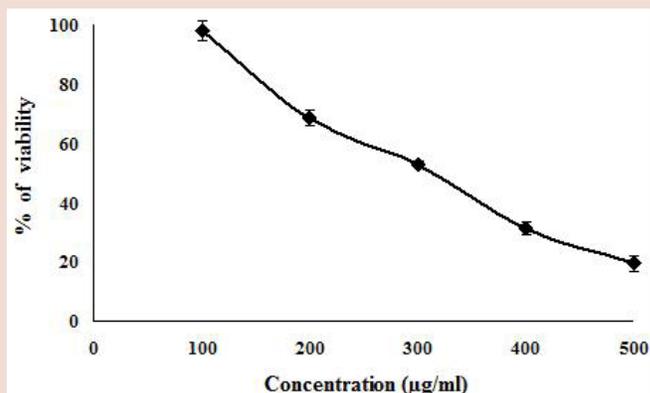


Figure 3: Cytotoxic activity of *P. odorata* methanolic extracts on Lung cancer (Calu-6) cell lines.

Extract were incubated with 10⁵ viable cells at concentrations ranging from 0 to 500 µg/ml for 48 h. Cell viability was determined by the MTT method.

in-vivo experiments among the three fractions, hydroalcoholic and ethyl acetate fractions significantly inhibited the ascites tumor in EAC bearing mice after 9 days treatment and the IC₅₀ values were 477.71, 654.57 and 339.20 µg/ml respectively.¹⁰

Table 1: IC₅₀ values of methanolic extract of *P. odorata*

Cell Lines	IC ₅₀ Value (µg/ml)
MD-MB-231	315.16±1.7
PC-3	340.12±4.2
Calu-6	395.16±3.7

Results represent means ± standard deviation (n = 3).

Preliminary phytochemical examination of ethanolic fraction of *P. odorata* has been reported to contain flavonoids and most number of flavonoids are known to possess remarkable antitumor activity.¹⁴ Although it is most accepted that the clear antitumor mechanism of flavonoids is not well known, they are reported to decrease the high glycolytic activity of tumor cells by inhibition of Na⁺ and K⁺ ATPase in the plasma membrane¹⁵ and this may be one of the reasons for the inhibition of tumor growth. Similarly in our experiments the observed significant cytotoxic effect on Human Breast cancer (MD-MB-231), Prostate cancer (PC-3) and Lung cancer (Calu-6) by *P. odorata* extract may be due to the action of flavonoids and other constituents present in the extract. Therefore, it will be interesting to understand the chemical composition and better understand the mechanism of action of the bioactive constituents of the extract for developing it as drug for therapeutic application.

CONCLUSION

The results of this study indicate anticancer activities for *P. odorata* extract. *P. odorata* showed significant cytotoxicity against MD-MB-231 and Calu-6, when compared to PC-3 cells. The potential anticancer activities of *P. odorata* extracts may be due to the presence of flavonoids and other constituents. The experimental evidence could be useful for validating the traditional use of plant as source of easily available effective anticancer agents to the people, particularly in developing countries. Future investigations will be interesting to find medicinal compound and also better understanding its mechanism of action against cancer.

ACKNOWLEDGEMENT

The authors are grateful to Department of Phytochemistry, G7 Synergon Private Limited, and the ICMR for providing financial support. DBL

thank Jain University for the constant support and encouragement for carrying out research aspects.

CONFLICT OF INTEREST

The author declares no conflict of interest.

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