



## ANTITUMOR POTENTIAL OF *PARKINSONIA ACULEATA* L. LEAVES EXTRACT ON B16F10 MICE MELANOMA CELL LINE

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### ABSTRACT

Flavonoids are very potential to act at all stages of the carcinogenesis. The present study evaluates their action on melanoma cell line. The results showed that flavonoids from *Parkinsonia aculeata* L. (Leguminosae) leaves possess cytotoxic activity against B16-F10 mice melanoma cell lines with IC<sub>50</sub> value of 15±0.01µg/ml. Treatment of cells with 20µg/ml flavonoid extract reduced melanoma cell viability. IR and GC-MS spectral analysis confirms the presence of apigenin in the extract which is an antitumor flavonoid.

**KEYWORDS:** Flavonoids, Melanoma, Cytotoxicity, Cell Viability, Apigenin.

### INTRODUCTION

Cancer is still a major cause of mortality and morbidity in developing as well as in developed countries. Melanoma is the most lethal and aggressive form of skin cancer. Overall survival rate has only improved slightly despite advances in surgery, radiotherapy and chemotherapy. Molecular target agents are currently being studied in all treatment settings including that of chemoprevention, which is defined as the use of natural or synthetic non-essential dietary agents to interrupt the process of carcinogenesis and to prevent or delay tumor growth (Alley MC *et al.*, 1988). The WHO has estimated that approximately 80% of the world's population depends on traditional medicines for meeting their

primary health care needs (Carmichael J *et al.*, 1987). The areas of cancer and infectious diseases have a leading position in utilization of medicinal plants as a source of drug discovery. Among FDA approved anticancer and anti-infectious preparations drugs of natural origin have a share of 60% and 75% respectively (Fabricant DS *et al.*, 2001).

Natural products and their derivatives contribute more than half of all clinically administered drugs (Galvez M *et al.*, 2003). They possess a significant position in drug discovery for treatment of cancer and other infectious diseases (Harborne JB, 1998). The plant *Parkinsonia aculeata* L. (Leguminosae) is commonly known as "Vilaytikikar." In the traditional medicinal system the leaf extracts are used for the treatment of hepatopathy, bacterial diseases, typhoid fever, diabetes and trypanosomiasis. The aerial parts of the plant have been used to treat diabetes-related complications (Hsan KM *et al.*, 2010).

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## MATERIALS AND METHODS

### Collection of plant material

*Parkinsonia aculeata* L. (Leguminosae) were collected from Hoshangabad road, Bhopal, M.P., India. After identification of plant by Dr. Jagrati Tripathi HOD, Department of Botany, Unique College, Bhopal (M.P.) voucher specimen no.SSLJ/2008/PS/01 was procured in herbarium record maintained at the Laboratory, P.G. Department of Zoology, S.S.L. Jain P.G., College, Vidisha (M.P.). The plant material was thoroughly washed with water and was kept for drying in shade at room temperature for 20days. The thoroughly air dried plant material was grinded to powder to 40-60 mesh size.

### Solvent Extraction

The plant powder was extracted with soxhlet apparatus using ethyl acetate for 38 hrs (Harborne, 1998). The crude filtered through Whatman filter paper no.1. Filtrate was then evaporated to dryness on rotary vacuum evaporator. Silica Gel (60-120 mesh) Column purified fraction yielded yellow crystalline compound which was further tested for its bioactivity.

### Cell culture

The B16F10 melanoma cell line was kindly provided by NCCS Pune, India. Cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM), supplemented with 10% fetal bovine serum (FBS) and streptomycin plus penicillin (100µg/ml and 100IU/ml, respectively). Cells were cultured in a 5% CO<sub>2</sub> humidified atmosphere at 37°C until confluence. All the processes were carried out in a vertical laminar air flow chamber.

## EXPERIMENTAL PROTOCOL

### *In vitro* cell viability

#### Trypan Blue Exclusion Assay:

The percentage of viable and non viable cells was determined using trypan blue exclusivity stain. Cell growth and viability was measured by adding 0.4% trypan blue in 0.9% saline to a 50% dilution and cells were counted using hemacytometer. Cells were examined and counted in duplicates under light binocular microscope 100× (Olympus, Japan). Percentage cell viability was calculated by the formula:

$$\text{Cell viability} = \frac{\text{No. of viable cells}}{\text{Total No. of cells}} \times 100$$

#### Cell viability Quantification: MTT assay

The cultures were incubated in 96 well plates with 200µl of fresh supplemented medium and 50µl of

MTT (8mg/ml) for 4hr at 37°C and 5% CO<sub>2</sub>. After centrifugation (240g for min) to carefully remove the medium and non metabolized MTT, 100 µl of DMSO were added to each well to solubilize the MTT formazan produced by the cultured cells. After shaking for 30 min at room temperature, the plates were read with a Multiscan MCC/340 P spectrophotometer using 570nm for the reading and 690nm for the reference wavelength (Koehn FE *et al.*, 2005; Leite ACR *et al.*, 2007; Shukla Y *et al.*, 2004).

### Statistical Analysis

The experiments were performed in triplicate. The data regarding cell proliferation and viability assay were expressed as mean ± standard deviation. The values of IC<sub>50</sub> were calculated by R<sup>2</sup> equation. A P value less than 0.05 were considered significant.

Plants are promising sources of anticancer therapeutics. The effect of flavonoid extract from *P. aculeata* L. (Leguminosae) leaves was investigated *invitro* on B16-F10 mice melanoma cell lines using Cytotoxicity activity MTT assay and Trypan Blue exclusion test of cell viability.

The flavonoid extract possess high cytotoxic activity and inhibited the cell growth with IC<sub>50</sub> value of 15±0.01µg/ml concentration (Fig-1). Percentage viability of flavonoid extract is shown in Fig-2 which showed decrease in percent cell viability as compared to that of melanoma cell line control with 63.7% viability at 20µg/ml as compared to standard drug which is 45.5% at 500nM. It can be concluded that the flavonoid extract possess good antitumor activity in a dose dependent manner with concentration range 0.01-100µg/ml. The IR (Fig-3) and GC-MS (Fig-4) spectral analysis confirms the presence of apigenin in the flavonoid extract of *P. aculeata* L. leaves.

## DISCUSSION

The search for anticancer agents from natural sources has been successful worldwide. Active constituents have been isolated and nowadays are used to treat human tumors (Jing S *et al.*, 2007). The ethnopharmacological knowledge is helpful to lead the search for plants with potential cytotoxic activity (Thi-Shan D *et al.*, 2008). Melanoma is the most lethal and aggressive form of skin cancer. Available treatment for metastatic melanoma is still poor in overall response and survival. New treatment options as single or in combinations with the use of natural phytochemicals or herbal medicines may provide breakthrough results in the treatment of malignant melanoma.

## RESULTS

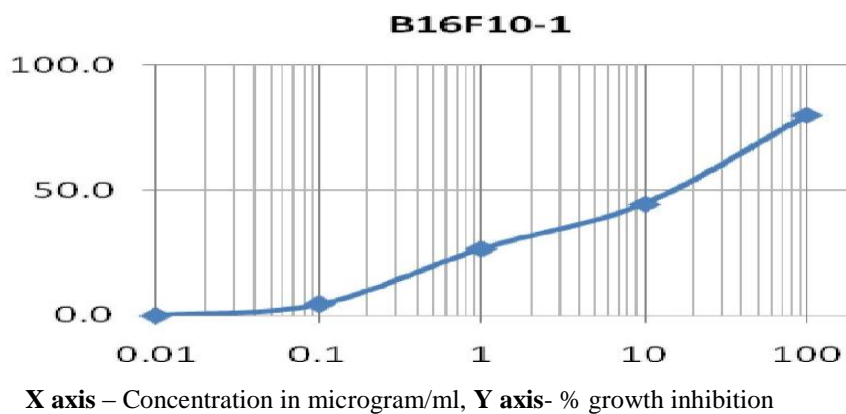
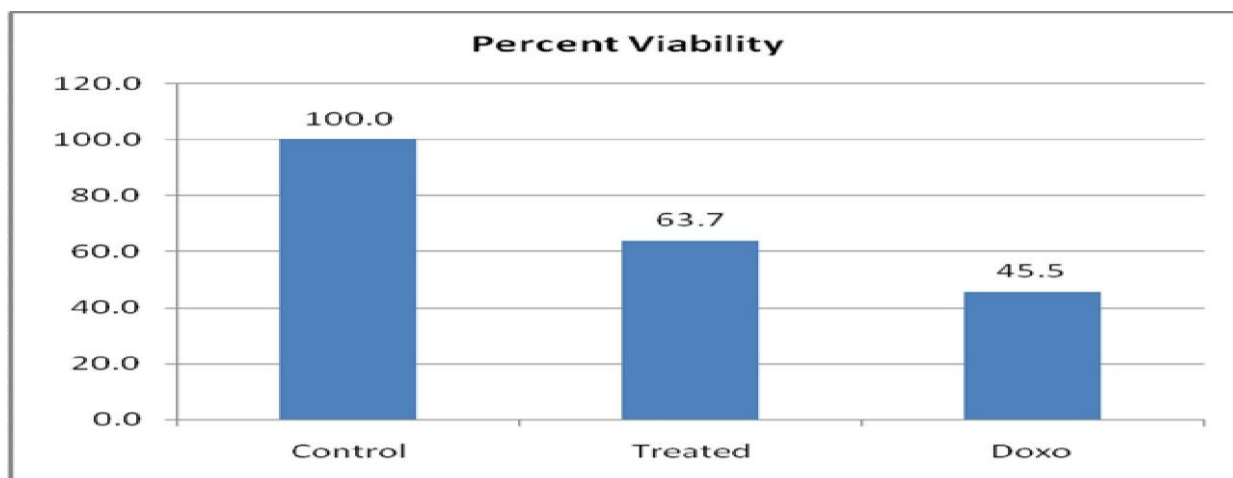
Fig 1. Dose Response Curve for Compounds against *B16F10* Cell LineFig 2. Trypan Blue Exclusion Assay Result on *B16-F10* Cells

Fig 3. Showing IR of the column purified fraction-III

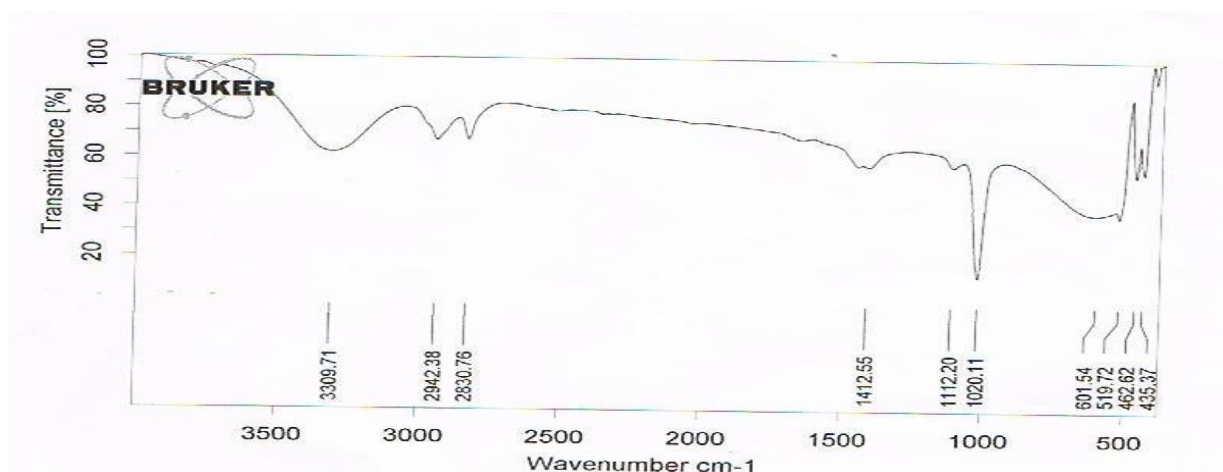
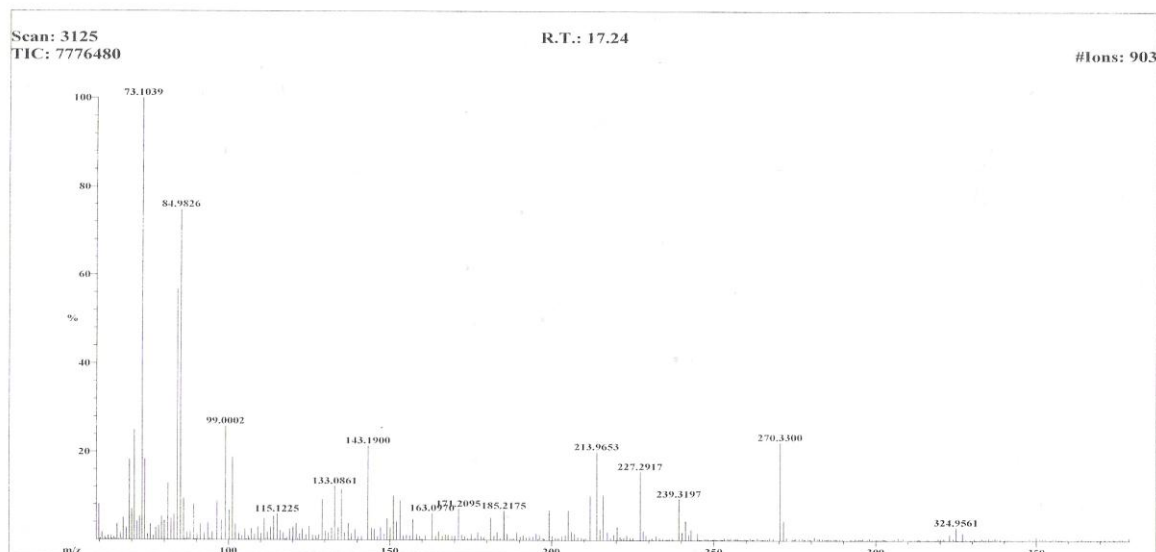


Fig 4. Showing GC-MS of the column purified fraction-III



## CONCLUSION

Our results demonstrate that flavonoid from *P. aculeata* L. leaves have growth inhibitory and cytotoxic effects on mice melanoma cell lines. Thus, this provides a scientific validation for further use of the plant extract for treatment of melanoma.

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