

## Phytochemical Screening ,Antimicrobial & Anti Proliferative Properties of *Rhododendron ponticum* on Prostate Epithelial Cancer Cells

ManiKumar P.<sup>1</sup>, Pardhu G.<sup>2</sup>, Shruti Ravinder<sup>2</sup>, Vijya Lakshmi<sup>2</sup>, Tejaswini K.<sup>1</sup> & Vishwanath Pradeep B\*.<sup>1</sup>

<sup>1</sup> Human Genetics Lab, Department of Zoology, Osmania University, Hyderabad - 500007, AP, India.

<sup>2</sup> Department of Biotechnology, Chaitanya Bharathi Institute of Technology, Gandipet, Hyderabad-500075, A.P, India.

### Abstract

Natural products continue to play an important role in the discovery and development of new pharmaceuticals. Several chemical compounds have been extracted and identified from its species known as *Rhododendron ponticum* (*R.ponticum*). The present study is designed for phytochemical analysis of *Rhododendron ponticum* the antimicrobial activity and anti proliferative activity on Prostate epithelial cancer cell lines. The study of the plant has been showing good antimicrobial activity and plant also exhibiting anti cancer properties.

**Key Words:** Phytochemical Screening, Antimicrobial activity, Anti proliferative, Prostate Cancer Cells, *Rhododendron ponticum*.

### INTRODUCTION

*Rhododendron ponticum* is an annual herbaceous plant found in most places. However, the widespread use of antibiotics in human medicine and agriculture has caused serious problem of bacterial resistance (Beovic et al.,2006). Therefore, plant derived antimicrobial agents with high potency and low mammalian toxicity, useful for food preservation and human health, have gained special interest in recent decades (Smid, E. J.& Gorris, L. G. M (1999), Essawi, T.& Srour, M(2000) & Reische et al.,1998). Recent pharmacological interest has focused on its anti-allergy and antibacterial effects (Wu et al., 1991). Phytochemical studies of its composition have led to the identification of a number of terpenes, including sesquiterpene lactones and triterpenes (Wu et al., 1991, Bohlmann, F. & Chen, Z.L.(1984)) The former class contained the major active constituents contributing to the anti-allergy and anti-bacterial activities of the herb. Despite it is used in the Chinese folk medicine to treat naso pharyngeal carcinoma (NPC) (Cheng, J.H & Li (1998) & Zhang., (2000)). Besides, both the anti-nasopharyngeal carcinoma potential and the potent constituents of *R.ponticum* remain elusive. Sesquiterpene lactones, most widely distributed within the *Compositae*, have received considerable attention for their anticancer properties (Zhang et al., 2005). 6-*O*-angeloylenolin, a sesquiterpene lactone containing an,  $\beta$ -unsaturated cyclopentenone, isolated from *R.ponticum* was reported to induce apoptosis in HL-60 cells *in vitro* and inhibit the solid

cancer growth in Lewis lung cancer xenograft model (Li et al., 2008).

In the present investigation, we report the Phytochemical analysis of available in the plant *Rhododendron ponticum* by HPLC, anti-bacterial activity of the crude & Anti proliferative activity on prostate epithelial cancer cells(PC3).

### MATERIALS AND METHODS

*Rhododendron ponticum* plants were collected from botanical garden, Hyderabad and plants identity was confirmed by plant taxonomist.

#### Extraction and Analysis

Fresh flowers, stem, root & leaves, were collected, washed and weighed (10 g each). The materials were then macerated in 10 ml of water, methanol, acetone & benzene separately and then kept for 6 h at room temperature. The mixtures were then filtered through sterile Whatmann filter paper No.1. The filtrates obtained were then centrifuged at 5000 rpm for 5 min. The supernatants were collected in a beaker and the solvents were allowed to evaporate. Then the dry extracts were stored at 4°C. These extracts were dissolved in 1-3 mL (w/v) of dimethyl sulfoxide (DMSO) (Priya and Ganjewala 2007). The samples were further extracted by passing through the column of cotton, silica gel, activated charcoal and again silica gel in ratio 1:2:1 to obtain the extracts. Thus the collected extract was passed into the column, number of times, to obtain the pure compound.

### Phytochemical Screening

Specific qualitative tests were performed for detection of metabolites in leaf and flower extracts. Alkaloids were estimated from previously published procedures (Clarke and Williams 1955). The presence of sterols was confirmed by the addition of 2 ml of acetic anhydride to 0.5 g of dried ethyl acetate extract with 2 ml of concentrated sulphuric acid. For the identification of phenolics, one ml of neutral ferric chloride was added to one ml of the extract. For the identification of terpenes, the extracts were treated with tin and thionyl chloride. For the identification of Flavones, 10 % sodium hydroxide was added. To reveal the presence of tannins, 0.5 g of the dried powder of the leaves and flowers were boiled with 5 ml of water in a test tube and then filtered. To the filtrate, ferric chloride was added and kept undisturbed for the observation. Phospholipids and glycolipids were estimated based on previously published procedures (Roughan and Batt 1969, Lowry and Tinsley 1976). To reveal the presence of Fixed oils, small quantity of petroleum ether and benzene extract was pressed separately between two filter papers.

#### ANTI BACTERIAL ACTIVITY

##### Pour Plate Technique

Nutrients required for the growth of micro organisms were taken into a 250ml conical flask and 100 ml of distilled water was added. pH was adjusted to 7.2 and 2 gm of agar was added. Then the nutrient agar medium was sterilized in an autoclave at 121°C under 15 lbs pressure for 15-20 minutes.

The bacterial strains were collected from microbial type culture collection (MTCC) of IMTECH, Chandigarh. *Escherichia coli*, *Pseudomonas aeruginosa*, *Bacillus subtilis* and *Streptococcus aureus*.

Pure cultures nutrient agar plates were prepared by taking a loopful of culture from stock cultures and it was streaked on Petri plates in streak plate method to obtain fine isolated colonies. The petriplates were incubated at 37°C for 24 hrs.

Pour plates allow for the growth of isolated colonies on the surface of the agar. The standard procedure, agar well diffusion method, was followed to test the antibacterial activity by pour plate method (Deena and Thoppil 2000). A loopful of inoculum containing the microorganism from the broth was poured on a sterile agar medium plate. Again the loop was sterilized on the flame and continued to pour the bacteria again. The plate was rotated for about 90° and the bacteria were spread. The

process was repeated as per the requirement. Later the plates were incubated. The crude and column cleaned up HPLC compound were used to test the antibacterial activity by disc method. The plates were observed for inhibition of culture growth by the tested compounds.

#### ANTI CANCER ACTIVITY

##### Cell Lines and culture conditions:

The anticancer activity of the extracted compound was studied by the cell viability and morphological analysis. Human Prostate Aden carcinoma epithelial Cancer cell line (PC3) were collected from National centre of cell science Pune and routinely cultured in *Dulbecco modified eagles* medium. Culture medium preparation was carried out by taking precautions of bacterial and fungal contaminants. By changing volume of Trypsin/EDTA we can bring the cultures cell lines in to adherent and semi adherent suspensions and they are used directly. Aliquot of cells (100-200µl) are removed and performed cell count and the cell viability should not excess 90% in achieve good recovery after freezing and they resuspended in to freezing medium.

##### CryoPresevation of Cell line

Cultures were observe using an inverted microscope to assess the degree of cell density and confirm the absence of bacterial and fungal contamination adherent and semi adherent cells were brought in to suspension using trypsin / EDTA and were resuspended in a volume of trypsin. Suspension cell lines can be used directly.

##### Cell Treatment

*Rhododendron ponticum* extracted compounds obtained by column clean up were used to evaluate anticancer effect. These compounds were prepared as 10mM stock solution in 100 % DMSO and were stored in dark colour bottle at 4°C. The cells are exposed to drug individually for a period of 48 hrs. Cells grown in the medium containing equivalent amount of DMSO without drug serve as control.

#### RESULT AND DISCUSSION

The pink color appearance when crude extract is treated with tin and thionyl chloride indicates the presence of terpenes. The change in color from yellow to orange when the crude extract is treated with 10% NaoH shows the presence of Flavones. Oil stained on paper when benzene extract treated with petroleum ether indicates the presence of fixed oil. Appearance of cream color when treated with Mayer's reagent indicates the presence of an alkaloid (Table 1).

**Table 1:** Phyto Chemical screening of *Rhododendron ponticum*

Qualitative Test	<i>Rhododendron ponticum</i>
Terpenes	+ ++
Fixed Oils	+
Flavones	+++
Alkaloids	++

+: Low, ++: Medium & +++: High concentration

**Anti-Bacterial Activity**

The order of susceptibility of *Rhododendron ponticum* plant extract on microorganisms is as follows: P.aeruginosa > B.subtilis > S.aureus> E.coli( Table 2).

**Table 2:** Anti-Bacterial activity of crude and column extracts *Rhododendron ponticum*

Microbes	Zone of Inhibition in mm(Mean ± SD)	
	Crude	Column
E.coli	7.6±0.6	4.4±0.6
S.aureus	7.7±0.8	4.5±0.2
B.subtilis	7.2±1.2	5.8±0.8
P.aerogens	8.4±0.4	5.6±0.8

Bioactive potential of Flavonoids has been reported. (Illic et al., 2004, Cushner and Lamb 2005).The present results are compared with Samy and Ignace Muthus (1999) & Sanchez et al (2005). Based on the fact that micro organism are becoming resistant against the drugs in use, present investigation is of great importance in pharmaceutical industries for preparing plant based antimicrobial drugs.

**AntiCancer Properties:**

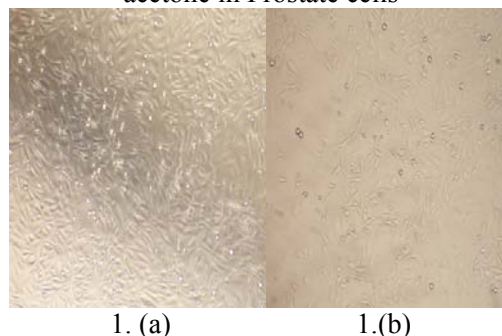
The reduction in overall tumor incidence can be attributed to ability of these compounds to interfere in the initiation of carcinogenesis and thereafter promotion of tumors.(Jagdeep et al.,2008).

The present results are comparable to that of *I. viscosa* leaf extract induced cytogenetic alterations (cytoplasmic shrinkage, nuclear condensation, DNA fragmentation, membrane blebbing, cytoskeleton alterations and appearance of apoptotic bodies) aid mainly cell death in root tips of *A. cepa* (Tulay et al.,2010).The present results are useful for designing novel therapeutc agents

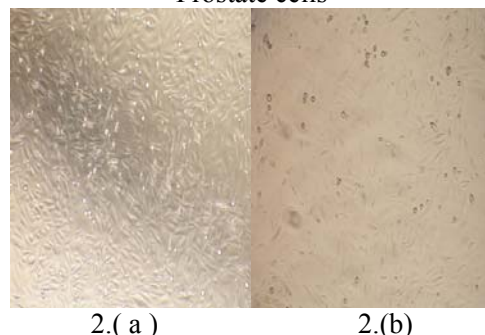
**Table 3:** Prostate cancer cell (PC3) death percentage of *Rhododendron ponticum*.

S.No	Sample	Solvents	Cell Death
1	Control		100%
2	Root	Water	8.49%
3	Stem	Water	7.86%
4	Leaf	Acetone	91.18%
5	Flower	Water	7.76%

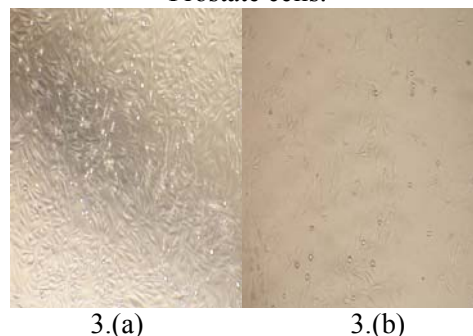
**Fig: 1:** Control compared with leaf extract with acetone in Prostate cells



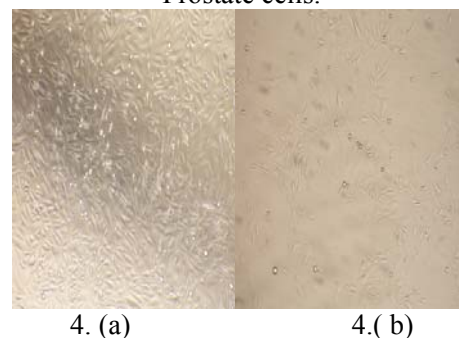
**Fig: 2:** Control compared with flower in water in Prostate cells



**Fig: 3:** Control compared with stem in water in Prostate cells.



**Fig: 4:** Control compared with root in Water in Prostate cells.



The figures 1(a),2(a),3(a)& 4 (a) ,represents the Control of Cells have only Prostate cancer Cells. The fig 1(b) represents the leaf extract in Acetone exhibiting more anticancer properties than stem in water [3(b)], Flower in water [2(b)] and Root in water [4(b)]

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#### REFERENCES

- Beovic, B. The issue of antimicrobial resistance in human medicine. *Int. J. Food Microbiol.* **112**: 280-287(2006).
- Bohlmann, F.; Chen, Z.L. New guaianolides from *Rhododendron ponticum*. *Chin. Sci. Bull.* **29**:900-903(1984).
- Cheng, J.H.; Li, Y.B. Anti-tumor Herbal Medicines and Their Proved Recipes; *Jiangxi Science &Technology Press*: Jiangxi, China,p. 732(1998).
- Clarke, E. G. C. and Williams, M.: Microchemical Tests For The Identification Of Alkaloids. *Journal of Pharmacy and Pharmacology* **7**: 255-262 (1955).
- Cushner TP, Lamb AJ:Antimicrobial activity of Flavonoids *Int. J.Antimicrobes Agents* **26(5)**: 343-356(2005).
- Deena MJ, Thoppil JE :Antimicrobial activity of the essential oil of *Lantana camara*. *Fitoterapia* **71**: 453-455(2000).
- Essawi, T.; Srour, M. Screening of some Palestinian medicinal plants for antibacterial activity. *J.Ethnopharmacol.***70**: 343-349(2000).
- Ilic SB, Konstantinovic SS, Todorovic ZB: Antimicrobial activity of bioactive component from flower of *Linum capitatum* kit. *Facta Universitatis* **3(1)**: 73-78(2004).
- Jagdeep Kaur , Manu Sharma P, D. Sharma.Chemopreventive activity of lantadenes on two-stage carcinogenesis model in Swiss albino mice: AP-1 (c-jun), NFjB (p65) and P53 expression by ELISA and immunohistochemical localization. *Mol Cell Biochem.* **314**:1–8(2008).
- Jane A, Plumb :Cell sensitivity assays: The MTT assay ,*Methods in Molecular Medicine*, Volume **88(4)**, 165-169, (2004).
- Lee K-H.,Ibuka T.,Wu, R-y & Giessman,T.A.Strutural antimicrobial relationships among the sesquiterpene lactones and related compounds.*Phytochemistry* **16**:1171-1181
- Li, C.L.; Wu, H.Z.; Huang, Y.P.; Yang, Y.F.; Liu, Y.W.; Liu, J.W. 6-O Angeloylenolin induces apoptosis through a mitochondrial/caspase and NF-κB pathway in human leukemia HL60 cells. *Biomed. Pharmacother.***62**:401-409(2008).
- Priya K, Ganjewala D: Antibacterial Activities and Phytochemical Analysis of Different Plant Parts of *Nyctanthes arbor-tristis* L. *Research Journal of Phytochemistry* **1**: 61-67(2007).
- Reische, D. W.; Lillard, D. A.; Eintenmiller, R. R. Antioxidants in food lipids. In *Chemistry,Nutrition and Biotechnology*; Ahoh, C. C.; Min, D. B. (Eds.); New York: Marcel Dekker, pp. 423-448(1998).
- Roughan PC, Batt RD: The glycerolipid composition of leaves. *Phytochemistry* **8**:363-369(1969).
- Samy PR, Ignacimuthus S, Raja DP.: Preliminary screening of ethnomedicinal plants from India. *J.Ethanopharmacol* **66(2)**: 235-240. (1999).
- Sanchez SRP, Kantun SP, Tapia TWS, Pat FM, Polanco SP, Rivera RC.: Screening of native plants from Yucatan for anti- Giardia lamblia activity. *Pharm Biol* **43(7)**: 594-596(2005).
- Smid, E. J.; Gorris, L. G. M. Natural antimicrobials for food preservation. In *Handbook of Food Preservation*; Rahman, M. S. (Ed.); New York: Marcel Dekker, pp. 285-308(1999).
- Tulay Aşkin Çelik and Ozlem Sultan Aslanturk:Evaluation of Cytotoxicity and Genotoxicity of *Inula viscosa* Leaf Extracts with Allium Test. *J Biomed Biotechnol.* 189252 **(2010)**.
- Wu, J.B.; Chun, T.T.; Ebizuka, Y.;Sankawa, U. Biologically active constituents of *Rhododendron ponticum*: Sesquiterpenes of potential anti-allergy activity. *Chem. Pharm. Bull.* **39**: 3272-3275(1991).
- Zhang, Y.H. A Collection of Anticancer Chinese Medicines. *Jiangsu Science and Technology Publishing House*: Nanjing, China, ; p. 435(2000).
- Zhang, S.; Won, Y.K.; Ong, C.N.; Shen, H.M. Anticancer potential of sesquiterpene lactones:bioactivity and molecular mechanisms. *Curr. Med. Chem. Anti-Cancer Agents*:**5**: 239-249(2005).