Int. J. Chem. Sci.: 10(1), 2012, 317-323
ISSN 0972-768X
www.sadgurupublications.com

IN VITRO ANTICANCER ACTIVITY OF ETHANOLIC EXTRACTS OF *PERISTROPHE BICALICULATA NEES*

SANGEETA S. TANAVADE*, NILOFER S. NAIKWADE and DHANYAKUMAR D. CHOUGULE

Appasaheb Birnale College of Pharmacy, SANGLI – 416416 (M.S.) INDIA

ABSTRACT

In the present study, *in vitro* anticancer activity of ethanolic extracts of leaves and stems of *Peristrophe Bicaliculata*, *Nees* were evaluated. In vitro anticancer activity was assayed by tryphan blue dye exclusion method and brine shrimp lethality bioassay method. Extracts were found to be cytotoxic in a dose dependent manner in both methods. The CTC_{50} in leaves is 165 µg/mL and in stems is 219 µg/mL in EAC cell lines. The LC_{50} in leaves is 173 µg/mL and in stems is 207 µg/mL in brine shrimps. Concentration needed for 50% inhibition was found to be less in leaves as compared to stems. It indicates that *Peristrophe Bicaliculata*, *Nees* has potential to prevent cancer in a dose dependent manner.

Key words: *Peristrophe Bicaliculata*, Cytotoxicity, Anticancer, EAC cells.

INTRODUCTION

Approximately 1.3 million cases of cancer are diagnosed each year in India resulting in an estimated 5, 70, 280 annual deaths. Cancer is a second leading cause of death after heart disease. Thus cancer is a serious clinical hazard that possesses significant social and economic challenges to healthcare system. Most anticancer drugs currently used are cytotoxic to normal cells and cause immunotoxicity, which affect not only tumor development but also aggravates patient's recovery.

Discovery of antitumor drug with less toxic effects has become an essential goal. Plants can serve as a prime source for achieving this goal for treatment of various forms of cancer. *Peristrophe Bicaliculata*, *Nees* Family- Acanthaceae is an erect perennial herb 60-120 cm height found in forest undergrowth, hedges and waste band native to warm temperate and tropical regions throughout India, Afghanistan and Africa. The plant is used as a remedy for snake bite. The whole plant is used to alleviate consumption. The leaves of

*Author for correspondence; E-mail: tan.sangeeta@gmail.com

the plant used traditionally as analgesic, antipyretic, anti-inflammatory, anticancer, sedative, diuretics and to treat diarrhea. The 50% hydro ethanolic extract of plant showed good anti inflammatory and analgesic activity. (Rathi et al., 2003). The aqueous extract of plant also showed anti inflammatory analgesic and antimicrobial activity. (Qureshi et al. 1977, Chopra and Chopra, 1959).

EXPERIMENTAL

Plant material

The plant *Peristrophe Bicaliculata, Nees* was collected from area around Sangli region during the month of September. The plant was identified and authenticated by Dr. (Mrs.) U. S. Yadav, Willingdon College, Sangli.

Preparation of the extract

From the collected plant material leaves and stems were separated. They were crushed, dried separately into shade at room temperature. They were separately powdered and sieved through No. 20 mesh sieve. The successive solvent extraction was carried out using Soxhlet apparatus. About 50 g of powder was extracted with 400 mL of solvent. Aqueous extract was prepared by macerating the drug in water containing 4% chloroform. The extracts were dried by using rotary vacuum evaporator. Ethanolic extract of leaves and stems of *Peristrophe Bicaliculata* were used for screening in vitro anticancer activity. The *in vitro* anticancer activity was performed by two methods, tryphan blue dye exclusion method and brine shrimp lethality bioassay method.

In vitro cytotoxicity assay by tryphan blue dye exclusion technique.

Chemicals: Phosphate buffer saline, 5 fluro Uracil, Dimethyl sulphoxide (DMSO), Tryphan blue dye. **Equipments-** Carbon dioxide (CO_2) incubator, Haemocytometer.

Cell lines: Ehrlich ascites carcinoma (EAC) cells –purchased from National centre for Cell Sciences, Pune, India.

Procedure: Samples of extracts were prepared by dissolving 20 mg of extract in 40 µL of DMSO then making the volume with phosphate buffer saline to make 1000 µg/mL stock solution. From this stock solution different drug concentrations, 50 µg/mL, 100 µg/mL, 200 µg/mL, 500 µg/mL were prepared. 5 fluro Uracil was used as a standard. The ascitic fluid was aspirated from the peritoneal cavity of tumor bearing mice. It is mixed with phosphate buffer saline. It is centrifuged and cells were washed with phosphate buffer saline. Then it is transferred to 24 wells micro plates and 100, 150 and 200 microlitre doses of

extracts were added in plates. The plates were then incubated in CO_2 incubator for three hours at 37°C. Then 0.1 mL tryphan blue was added. The total number of dead cells and living cells were counted by using Haemocytometer and percent cytotoxicity was calculated.

Brine shrimp lethality bioassay

Chemicals: Brine shrimp eggs (Arteria Salina): purchased from Central Institute of Fisheries Education (CIFE), Mumbai. Crude sea salt,

Equipments: Hatching chamber- fabricated as per design Meyer et al. 1982.

Procedure: The eggs of Brine shrimp were sprinkled in hatching chamber containing sea water. After 48 hours the eggs got hatched. Phototropic nauplli were seen. Samples of extracts were prepared by dissolving 20 mg of extract in 40 µL of DMSO then making the volume with phosphate buffer saline to make 1000 µg/mL stock solution. From this stock solution, different drug concentrations, 50 µg/mL, 100 µg/mL, 200 µg/mL, 500 µg/mL were prepared. 5 fluro Uracil was used as a standard. The phototropic nauplli were collected by capillary and ten of such shrimps were transferred to each sample vial containing brine solution (specific volume brine and yeast suspension). Then the plant extracts of different concentrations were added to different vials. The vials were maintained under illumination. Survivors were counted after 24 hours by using 3x magnifying glass and LC_{50} value was calculated.

RESULTS AND DISCUSSION

Table 1: *In vitro* anticancer activity of *Peristrophe Bicaliculata* against EAC cells by tryphan blue dye method

Sample	Concentration	Percentage cytotoxicity on EAC cell lines (Mean ± SEM)	CTC_{50}
Ethanolic extract of leaves of *Peristrophe Bicaliculata*	50 µg/mL	32.40 ± 0.2588	165.39 ± 12.23
	100 µg/mL	48.76 ± 0.3530	
	200 µg/mL	60.46 ± 0.4632	
	500 µg/mL	98.92 ± 0.3153	
DMSO	0.2% w/v	93.357 ± 0.6179	

Cont…

Sample	Concentration	Percentage cytotoxicity on EAC cell lines (Mean ± SEM)	CTC_{50}
5 Fluro uracil	20 µg/mL	33.943 ± 0.5937	
Ethanolic extract of stems of *Peristrophe Bicaliculata*	50 µg/mL	20.18 ± 0.701	219.87 ± 5.64
	100 µg/mL	32.04 ± 0.4654	
	200 µg/mL	45.48 ± 0.479	
	500 µg/mL	85.80 ± 0.4690	
DMSO	0.2% w/v	93.357 ± 0.6179	
5 Fluro uracil	20 µg/mL	33.943 ± 0.5937	

P < 0.05, Statistically significant when compared with positive control (5-fluro uracil) by ANOVA followed by student Newman-Keuls test

Table 2: *In vitro* anticancer activity of *Peristrophe Bicaliculata* by Brine shrimp lethality bioassay method

Sample	Concentration	Percentage cytotoxicity on brine shrimps. (Mean ± SEM)	LC_{50}
Ethanolic extract of leaves of *Peristrophe Bicaliculata*	50 µg/mL	35.55 ± 1.110	173.10 ± 9.76
	100 µg/mL	45.55 ± 1.110	
	200 µg/mL	57.77 ± 2.223	
	500 µg/mL	95.55 ± 1.110	
DMSO	0.2% w/v	93.357 ± 0.6179	
5 Fluro uracil	20 µg/mL	33.943 ± 0.5937	
Ethanolic extract of stems of *Peristrophe Bicaliculata*	50 µg/mL	21.11 ± 1.110	207.38 ± 12.23
	100 µg/mL	34.44 ± 1.110	
	200 µg/mL	48.23 ± 2.220	
	500 µg/mL	85.55 ± 1.110	
DMSO	0.2% w/v	93.357 ± 0.6179	
5 Fluro uracil	20 µg/mL	33.943 ± 0.5937	

P < 0.01, Statistically significant when compared with control by ANOVA followed by Dennett test.

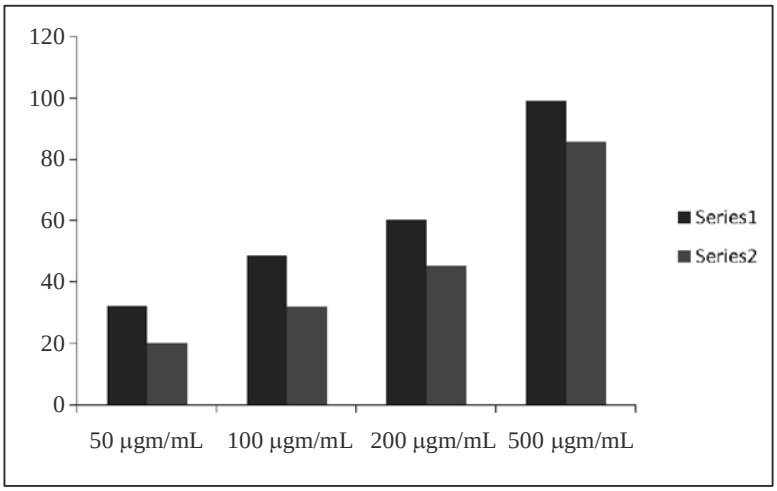

Fig. 1: *In vitro* anticancer activity by tryphan blue dye method

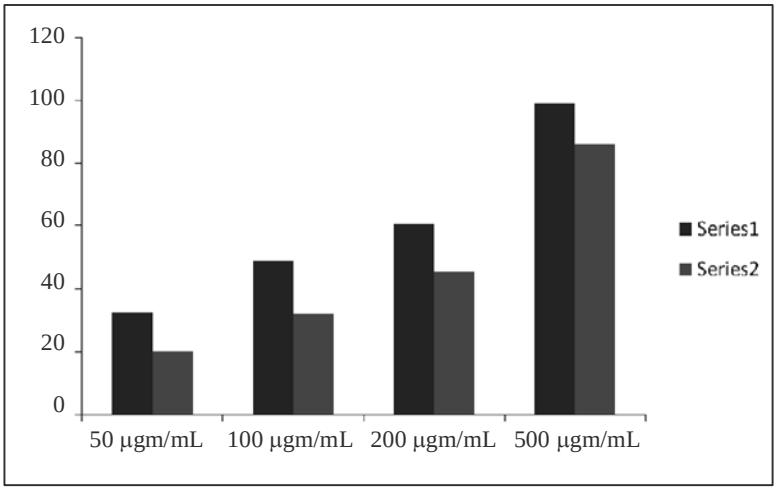

Fig. 2: *In vitro* anti cancer activity by brine shrimp lethality bioassay method

Series 1- Ethanolic leaves; Series 2- Ethanolic stem

Cancer is a leading cause of mortality worldwide and failure of conventional chemotherapy to effect a major reduction in mortality indicates that new approaches are critically needed. Here we have performed in vitro anticancer activity of ethanolic extract of leaves and stems of *Peristrophe Bicaliculata* by two methods, tryphan blue dye exclusion test and Brine shrimp lethality Bioassay method. In both methods, we have examined anticancer activity by giving four different doses, 50 µg/mL, 100 µg/mL, 200 µg/mL, 500 µg/mL respectively. Extracts were found to be cytotoxic in a dose dependent manner in both

methods. The Concentration needed for 50% inhibition for leaves and stems is 165 µg/mL and 219 µg/mL respectively by tryphan blue dye method. The Concentration needed for 50% inhibition is 173 µg/mL and 207 µg/mL, respectively by brine shrimp lethality bioassay.

CONCLUSION

Results of present study suggested that ethanolic extract of leaves and stems of *Peristrophe Bicaliculata* can induce tumor cell death. Potency of extract to bring cytotoxicity increases with dose. This confirms the folklore claim of usefulness of the plant in treating cancer. The present study is first time reporting in vitro anticancer activity of *Peristrophe Bicaliculata*. Though folklore claimed anticancer activity of leaves, present study reports that even stems of *Peristrophe Bicaliculata* have anticancer activity, but somewhat lesser than leaves.

ACKNOWLEDGEMENT

The author wish to thank Shri. D. D. Chougule, Principal, Appasaheb Birnale College of Pharmacy, for providing equipment and chemical facilities.

REFERENCES

1. P. Vijayan et al., *In vitro* cytotoxic Activity of Lanata Camara Linn, Indian J. Pharmacol., **36**, 94-95 (2004).
2. Y. Rajeshwar, M. Gupta and U. K. mazumdar, Antitumour Activity and *In Vivo* Antioxidant Status of Mucuna Pruriens Seeds Against Ehrlich Ascites Carcinoma in Swiss Alino Mice, Iranian J. Pharmacol. Therapeutics, **4**, 46-53 (2005).
3. B. Nicol and S. Prasad, The Effects of Cyclophosphamide Alone and in Combination with Ascorbic Acid Against Murine Ascites Dalton's Lymphoma, Indian J. Pharmacol., **38**, 260-265 (2006).
4. M. Gupta, U. Mazumdar, S. Chakraborti, S. Bhattacharya and N. Rath, Antiepileptic and Anticancer Activity of Some Indigenous Plants, Indian J. Physiol, Allied Sci., **51**, 53-56 (1997).
5. A. Jemal, R. Siegel, E. Ward et al., Cancer Facts and Figures 2008, American Cancer Society, Atlanta, 1002 (2008).
6. Patient Safety Advisory, *In Vitro* Hemolysis: Delays May Pose Safety Issues, PA-PSRS Patient Safety Advisory, 42-47 (2007).

7. W. Luttmann, K. Bratke, M. Kupper and D. Myrtek, Immunology, Elsevier (2006) p. 166-176.

8. K. D. Tripathi, Essentials of Medical Pharmacol. (2003) p. 785-792.

9. M. C. Bibby, M. Suggitt, 50 Years of Preclinical Anticancer Drug Screening: Empirical to Target-Driven Approaches, Clinical Cancer Res. (2005) p. 971-981.

10. H. Haslett, E. Chilvers, N. A. Boon and M. R. Colledge, Davidson's Principles and Practice of Medicine (2002) p. 217.

11. P. K. Haldar, M. Gupta, U. K. Muzumdar and C. C. Kandara, Anticancer Activity of Indigofera Aspalathoids and Wedelia Calendulaceae in Swiss Albino Mice, Iranian J. Pharmaceut. Res., **6**, 141-145 (2007).

12. M. S. Permila, Ayurvedic Herbs: A Clinical Guide to the Healing Plants of Traditional Indian Medicine, Howerth Press (2006) p. 226-227.

13. Z. Ronai, T. Minamoto and M. Mali, Environmental Factors as Regulators and Effectors of Multistep Carcinogenesis (1999) p. 519-527.

14. V. Subhadradevi, J. Christy, K. Ashok Kumar, M. Umamaheshwari and A.T. Sivashanmugham, Induction of Apoptosis and Cytotoxic Activities of Methanolic Extract of Careya Arborea Roxb. Bark, Pharmacie Globale (IJCP), **1** (2010) p. 1-3.

15. K. Zandi, S. Tajbaksh, I. Nabipour et al., *In Vitro* Antitumor Activity of Gracilaria Corticata Against Jurkat and Molt-4 Human Cancer Cell Lines, African J. Biotechnol., **9**, 6787-6790 (2010).

16. M. Shoeb, Anticancer Agents from Medicinal Plants, Bangladesh J. Pharmacol., **1**, 35-41 (2006).

Revised : 14.10.2011 *Accepted : 15.10.2011*