International Journal for Pharmaceutical Research Scholars (IJPRS)

V-3, I-2, 2014 ISSN No: 2277 - 7873

RESEARCH ARTICLE

Evaluation of Anticancer Activity of Extract of *Aphanamixis Polystachya* (Wall.) Parker Leaves

Karunakar Hegde*, Gargi Moitra

Department of Pharmacology, Srinivas College of Pharmacy, Valachil-574 143, Mangalore, Karnataka, India.

Manuscript No: IJPRS/V3/I2/00322, Received On: 30/06/2014, Accepted On: 04/07/2014

ABSTRACT

Cancer is a condition in which cells undergo uncontrolled proliferation which can be caused due to chemical agents, radiations or oxidative stress. Methanolic extract of leaves of *Aphanamixis polystachya* (MLAP) was studied for *in vitro* anticancer activity using T47D breast carcinoma cell lines and HeLa human cervical carcinoma cell lines and *in vivo* anticancer activity against EAC cell lines with two doses 200 mg/kg and 400 mg/kg. Radio protective activity of the extract was also studied on human breast carcinoma cell lines using UV radiation (15 W, 365 nm) with an exposure time of 15 min, with cell lines placed at a distance of 3 cm from the radiation. MLAP exhibited significant *in vitro* anticancer activity against T47D and HeLa cell lines and *in vivo* anticancer activity in EAC bearing mice with a potential of radio protection against UV radiation. These findings justify the traditional use of this plant in the treatment of cancer and validate its claim of being used for the said purpose in folklore claim.

KEYWORDS

Anticancer, *Aphanamixis Polystachya*, EAC Cell Lines, Radio Protective Activity

INTRODUCTION

Cancer is the uncontrolled growth and quick division of the abnormal cells in the body. These cells invade and destroy the surrounding tissues. Inspite of all advances in medical sciences, cancer a disease as old as mankind is globally a major health problem[1]. Recent reports from the International Agency for Cancer Research indicates that, approximately 12.7 million new cancer cases and 7.6 million cancer death were occurred in the less developed regions of the world. Projections are that by the year 2020, the incidence of cancer will increase by threefold, and that there will be a disproportionate rise in cancer cases and deaths from the developing countries that have resources to tackle the problem[2].

***Address for Correspondence:**
Karunakar Hegde
Department of Pharmacology, Srinivas College of Pharmacy, Valachil-574 143, Mangalore, Karnataka, India.
E-Mail Id: khegde_sh2003@yahoo.co.in

In the treatment of cancer many synthetic and chemotherapeutic agents have been developed, that show various side effects like alopecia, skin eruptions, reduced immunity, secondary carcinogenesis, etc. Hence, to overcome these flaws and to make the course of treatment more convenient herbal drugs have been developed as they are not known for severe side effects. There are good enough plants like *Catharanthus roseus*, *Amoora rohituka*, *Andrographis paniculata*, *Azadirachta indica*, *Bauhinia variegate*, etc are used by the local tribes for the cancer treatment.

One such plant, *Aphanamixis polystachya* (WALL.) Parker belongs to the family Meliaceae, locally known as Tiktaraj, a large tree grows wild and planted in forests and roadsides all over the country. The plant is extensively used in traditional system of medicine for various ailments like

spleenomegaly, liver complaints, tumors, ulcers, haemorrhoids, burning sensations, ophthalmia, nervousness and rheumatism[3].

The plant is reported to possess hepatoprotective[4], insecticidal, antibacterial, antifungal and immunosuppressive activities[5,6]. Further the stem and seeds extract were reported to possess anticancer activity[7,8]. The leaves of the plant are also used by tribal healers of Western Ghats region of India to ameliorate cancer. However, no scientific reports were found reporting anticancer activity of the leaves of *A. polystachya* hence, the present study was designed to evaluate the anticancer activity of methanolic extract of the leaves of *A. polystachya* against T47D human breast carcinoma cell line and HeLa human cervical cancer cell line to assess the *in vitro* anticancer activity and EAC (Ehrlich Ascites Carcinoma) induced liquid tumor model for *in vivo* anticancer activity. According to the literature claim the previous studies have reported that the bark extract of the plant also possesses radioprotective activity. Hence, the radioprotective potential of the methanolic extract of the leaves of *A. polystachya* (MLAP) was also tested using UV as the source of radiation.

MATERIALS AND METHODS

Collection of Plant Material and Extract

The leaves of *Aphanamixis polystachya were* collected from Vittala, Mangalore. The plant was identified and authenticated by the Botanist Mr. Dinesh Nayak, Adviser (Green Belt) Mangalore SEZ, Mangalore. The shade dried leaves were crushed and around 150 g of leaves were subjected to extraction by soxhletion with 500 ml of methanol as solvent. The extraction was continued until complete exhaustion of active constituents.

The total extract was filtered by using Whatman filter paper No.1 and concentrated to syrupy consistency and evaporated to dryness under reduced pressure at low temperature on a rotary flash evaporator to give methanolic extract. Yield of the drug product obtained was 5.7% as dried solid mass by weight[9].

Experimental Animals

Adult mice of either sex (25-30 g) were maintained under standard conditions (12 h light/dark cycle; 27±2°C, 45-60% relative humidity) and were fed standard chow and water *ad libitum.* All the animals were acclimatized to laboratory conditions for a week before commencement of experiment. All experimental protocols were approved by Institutional Animal Ethical committee (Approval No. SCP/CPCSEA/P01/F150/2011) prior to the initiation of the experiment.

Preliminary Phytochemical Investigation

Preliminary phytochemical tests were carried out to detect the presence of active constituents as per standard procedure[10].

Acute Toxicity Study

Acute toxicity study of methanolic extract of leaves of *Aphanamixis polystachya* was determined in Wistar albino rats according to OECD guidelines No. 425[11]. The animals were fasted overnight and the methanolic extract 2000 mg/kg was administered orally. Animals were observed continuously for first 3 h and monitored for 14 days for mortality and general behavior of animals, signs of discomfort and nervous manifestations.

In Vitro Anticancer Activity

MTT Assay

T47D Human breast carcinoma and HeLa human cervical carcinoma cell lines were plated in a 96 well microtiter plate and incubated for 48 h followed by which cells were treated with 1000 µg/ml, 500 µg/ml, 250 µg/ml, 125 µg/ml, 62.5 µg/ml, 31.25 µg/ml concentrations of the extract and compared with 500 µg/ml, 250 µg/ml, 125 µg/ml, 62.5 µg/ml, 31.25 µg/ml, 15.625 µg/ml of Cisplatin. The well was incubated for 24 h and then MTT assay was performed. Here, the dose efficacy was evaluated based on the cell viability by values represented as IC_{50} value[12,13].

In Vivo Anticancer Activity

EAC Induced Liquid Tumor Model

Animals were group into five groups (n=6). Group I, was normal without any treatment and tumor. Group II control, contained tumor induced animals without treatment. Group III contained tumor induced animals treated with 200 mg/ml extract. Group IV contained tumor induced animals treated with 400 mg/ml extract. Group V contained tumor induced animal being treated with Cisplatin. Evaluation was done on the basis of mean survival time (MST). Thus, the animals were observed for the duration of 25 days[14,15].

Radioprotective Activity

Ultraviolet light (15 W, wavelength 365 nm) was used as a source of radiation to which the cells were exposed for 15 min from a distance of 3 cm. and then the wells were treated with the 1000 µg/ml, 500 µg/ml, 250 µg/ml, 125 µg/ml, 62.5 µg/ml, 31.25 µg/ml concentrations of the extract was compared to the control wells, which contained only DMSO and media. Efficacy of radioprotective potential was evaluated by comparing cell viability by performing MTT and measuring the absorbance using ELISA reader at 540 nm[16,17].

Statistical Analysis

All values expressed as MST ± SEM and were analyzed by one way ANOVA (analysis of variance) followed by Dunnett's 't' test using SPSS 2010 software. P value less than 0.001 was considered significant.

RESULTS AND DISCUSSION

Preliminary phytochemical screening revealed the presence of alkaloids, glycosides, triterpenes, flavonoids, saponins and tannins.

There was no mortality and any signs of discomfort amongst the dosed groups of animals suggested that the MLAP is relatively safe in or non-toxic to experimental animals

The values of MTT assay were predicted using sigma plot by determining the IC_{50} value of the drug for different cell lines.

According to the IC_{50} value obtained (31.48 µg/ml), the methanolic extract at higher concentration showed a significant cytotoxic effect against HeLa human cervical carcinoma cell lines (Fig. 1) and IC_{50} value (115.84 µg/ml) showed a significant cytotoxic effect against T47D human breast carcinoma cell lines (Fig. 2).

Hence, the drug may be said to possess anticancer activity against human cervical carcinomas and human breast carcinomas.

In EAC induced liquid tumor model the activity of drug was assessed as per mean survival time (MST) and increase in life span (Table 1and Fig. 3).

It is observed that the methanolic extract of leaves of *A. polystachya* have better cytotoxicity at higher dose (400 mg/kg) as the mice treated with higher dose showed mean survival time (20.50, $p<0.001$) than that of the lower dose (200 mg/kg) treated mice (16.67, $p<0.001$).

Table 1: Effect of extract (MLAP) on EAC induced liquid tumor model

Animal No.	Control	MLAP (200 mg/kg)	MLAP (400 mg/kg)	Standard (Cisplatin)
1	10	17	20	25
2	15	16	21	25
3	8	17	19	25
4	10	18	20	25
5	9	16	20	25
6	10	16	23	25
SEM	0.99	0.33	2.81	0.00
% ST	0.00	38	49.59	58.67
Mean MST	10.33	16.66***	20.50***	25.00***

All values expressed as MST ± SEM and were analyzed by one way ANOVA followed by Dunnett's 't' test. ***P<0.001 when compared with control group.

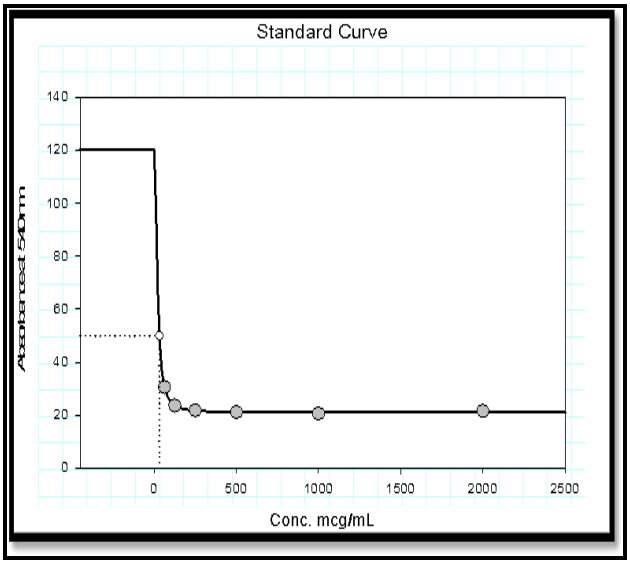

Figure 1: IC_{50} value (31.48 µg/ml) for HeLa human cervical carcinoma cell lines

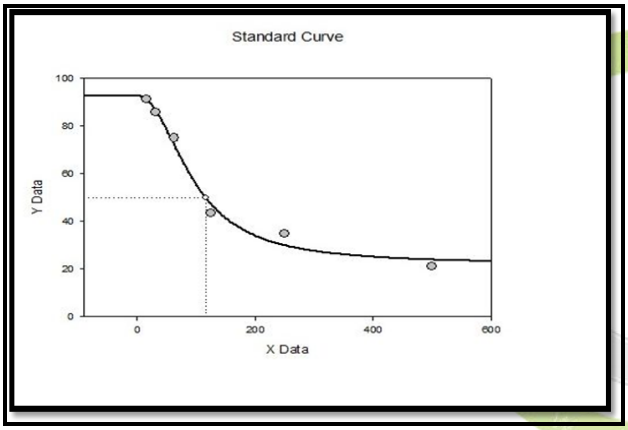

Figure 2: IC_{50} value (115.84 µg/ml) of T47D human breast carcinoma cell lines

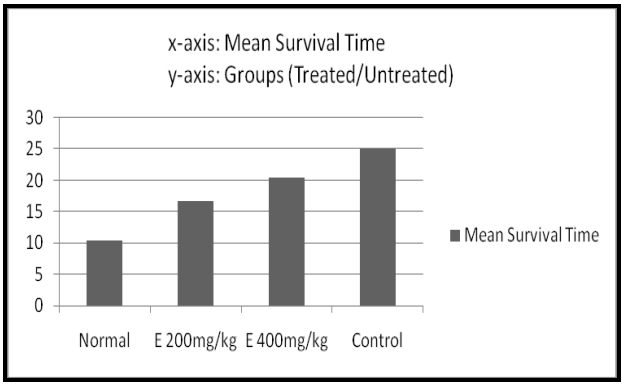

Figure 3: Mean survival time (MST) on EAC induced liquid tumor model

The radio protective effect of the methanolic extract of *Aphanamixis polystachya* was studied by subjecting the T47D (breast carcinoma) cell line to UV radiation, followed by treatment with the extract. The extract showed a remarkable radio protective activity at lower concentrations. This reveals that the drug is potent radio protective agent. MLAP was observed to possess a concentration dependent action with cytoprotective activity in lower concentration and cytotoxicity with higher concentration (Table 2 and Fig. 4).

Table 2: Effect of extract (MLAP) on cell viability by radio protective activity

MLAP Concentration	% Survival
7.812 µg/ml	88.65%
15.625 µg/ml	85.88%
31.25 µg/ml	75.17%
62.5 µg/ml	43.26%
125 µg/ml	34.75%
250 µg/ml	21.27%

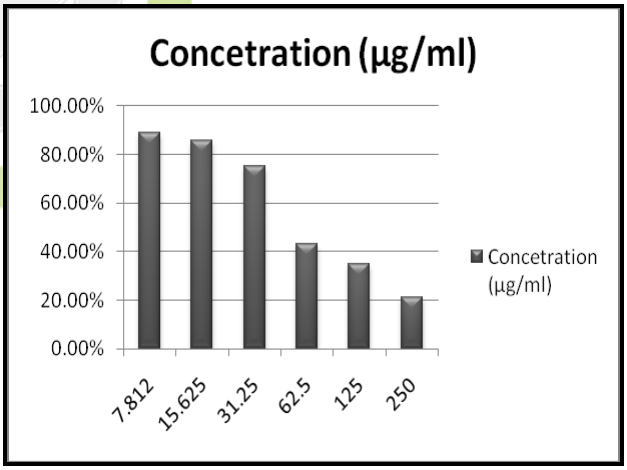

Figure 4: Effect of extract (MLAP) on cell viability by radio protective activity

Qualitative analysis for phytochemical investigation indicated the presence of alkaloids, glycosides, triterpenes, flavonoids, saponins and tannins. The literature survey enlightened that, members of family Meliaceae known contain certain types of limonoids, which are potent anticancer constituents. Saponins are the detergent molecules that alter the permeability

of the cell membrane, thus leaking the cellular components by rupturing the membrane. Alkaloids and other polyphenolic compounds act on nucleus by disrupting the mitotic spindles and arrest the cell cycle at the metaphase[18,19]. Therefore, these phytochemicals present in the methanolic extract of this plant may attribute to the potential anticancer activity.

CONCLUSION

It can be concluded that the data obtained in the present study suggest that the methanolic extract of the leaves of *Aphanamixis polystachya* possesses significant, dose dependent cytoprotective and cytotoxic effect. Further, it concludes that, the MLAP possess potential anticancer activity.

It is worthwhile to isolate the bioactive principles, which are responsible for these activities. The findings justify the traditional use of this plant in the treatment or control of cancer and validate its claim of being used for the said purpose in folklore medicine.

AKNOWLEDGEMENT

The authors are thankful to the authorities of A Shama Rao Foundation Mangalore, Karnataka, India for providing required facilities.

REFERENCES

1. Arora, R. (2010). Herbal Drugs: A Cancer Chemopreventive and Therapeutic Perspective. New Delhi: Jaypee Brothers Medical Publishers (P) Ltd.
2. Are, C., Colburn, L., Rajaram, S., & Vijayakumar, M. (2010). Disparities in cancer care between the United States of America and India and opportunities for surgeons to lead. *Journal of Surgical Oncology*, 102, 100-105.
3. Kirtikar, K. R., & Basu, B. D. (2004). Indian medicinal plants, Vol. 1, Dehradhun: Bishen Singh and Mahendra Pal Singh, 551-553.
4. Rao, K. N., Srinivasan, K. K., Udupa, A. L., & Krishanand, B. R. (1993). Anti-hepatotoxic activity of *Aphanamixis polystachya*. *Fitoterapia*, *64(6)*, 507-509.
5. Chowdhury, R., Hasan, C. M., Rashid, M. A. (2003). Antimicrobial activity of *Toona ciliata* and *Amoora rohituka*. *Fitoterapia*, *74(1-2)*, 155-158.
6. Srivastava, S. K., Srivastava, S. D., & Srivastava, S. (2003). New biologically active limonoids and flavonoids from *Aphanamixis polystachya*. *Indian Journal of Chemistry*, *42B(12)*, 3155-3158.
7. Rabi, T. (1996). Antitumor activity of amooranin from *Amoora rohituka* stem bark. *Current Science*, *70(1)*, 80-81.
8. Prasad, G. C. (1999). Critical care of cases with Ayurvedic drugs in the management of cancer. Patna: South-East Asian seminar on Herbs and Herbal medicines, 16-19.
9. Meheta, R. M. (2002). Pharmaceutics. Vallabh Prakashan, 3(1), 157-158.
10. Harborne, J. B. (1984). Phytochemical methods. A Guide to Modern Techniques of Plant Analysis. 2nd Ed, London: Chapman and Hall, 84-274.
11. OECD. (2001). Guidelines for testing of chemicals, Acute oral toxicity, Environmental Health and Safety Monograph Series on Testing and Adjustment No. 425, 1.
12. Mosmann, T. (1983). Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assay. *Journal of Immunological Methods*, *65(1-2)*, 55-63.
13. Freshny, I. R. (2006). Culture Of animal cells a manual of basic technique, Willey-Liss, 5, 366-368.
14. Jagetia, G. C., & Venkatesha, V. A. K. (2005). Enhancement of radiation effect by *Aphanamixis polystachya* in mice transplanted with Ehrlich ascites carcinoma. *Biological Pharma Bulletin*, *28(1)*, 69-77.
15. Yerra, Rajeshwar., Gupta, M., & Mazumder, U. K. (2005). Antitumor activity and in *vivo* antioxidant status of *Mucuna pruriens* (Fabaceae) seeds against Ehrlich Ascites

carcinoma in swiss albino mice. *Iranian Journal of Pharmacology and Therapeutics*, *4(1)*, 46-53.

16. Torsen, R. D., Fritz, G., & Kaina, B. (2001). Ultraviolet light-induced DNA damage triggers apoptosis in nucleotide excision repair-deficient cells via Bcl-2 decline and caspase-3/-8 activation. *Nature Oncogene*, *20*, 6026-6038.
17. Al-Mohanna, M. A., Al-Khodairy, F. M., Krezolek, Z., Bertilsson, P. A., Al-Houssein, K. A., & Aboussekhra, A. (2001). p53 dispensable for UV-induced cell cycle arrest at G_1 in mammalian cells. *Carcinogenesis*, *22(4)*, 573-578.
18. Wood, K. W., Cornwell, W. D., & Jackson, J. R. (2001). Past and future of the mitotic spindle as an oncology target. *Current Opinion in Pharmacology*, *1(4)*, 370-377.
19. Gigant, B., Wang, C., Ravelli, R. B., Roussi, F., Steinmetz, M. O., Curmi, P. A., Sobel, A., & Knossow, M. (2005). Structural basis for the regulation of tubulin by vinblastine. *Nature*, *435(7041)*, 519-522.

