Research article

In vitro anticancer activity of Ethanolic and Aqueous extract of *Putranjiva roxburghii* Wall

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ABSTRACT:

In the present study in vitro anticancer activity of ethanolic and aqueous extract of Putranjiva roxburghii Wall was evaluated. In vitro activity was assayed by MTT (3-[4, 5-dimethylthiazol-2-yl]-2, 5 diphenyl tetrazolium bromide). Ethanolic extract was found to be potent than aqueous extract. Leaves showed more significant anticancer activity. The IC_{50} of ethanolic extract was found to be 41.10% ug/ml in MCF-7 cell lines. The IC_{50} of aqueous extract of leaves was found to be 30.46 ug/ml. It indicates that *Putranjiva roxburghii* Wall has potential to prevent cancer dose dependent manner.

KEYWORDS: *Putranjiva roxburghii* Wall, anticancer, MTT (3-[4, 5-dimethylthiazol-2-yl]-2, 5 diphenyl tetrazolium bromide), Michigan Cancer Foundation-7 (MCF-7) cells.

INTRODUCTION:

Millions of people are affected by illnesses like cancer each year. Cancer is a group of diseases characterized by aberrant cell proliferation and the capacity to infiltrate or spread to various body parts. Changes in bowel habits, chronic coughing, tumors, and unexplained weight loss are some of the symptoms associated with cancer. 10.3 million cancer-related deaths and 19.3 million new cases of the disease will occur globally by 2020.[1]

The genetic changes found in cancer have a special effect on the two major categories of genes. Oncogenes that drive cancer are activated in cancer cells, endowing them with novel traits such as rapid growth and division, boundaries, and the ability to invade many tissue environments. Tumor suppressor gene inactivation causes cancer cells to lose their capacity to carry out normal functions such as accurate DNA replication, control over the cell cycle, orientation and adherence within tissues, and communication with immune system defense cells.[2]

This promoted our interest to study the anticancer activity from plant *Putranjiva roxburghii* Wall. Belonging to family Euphorbiaceae.

The plant *Putranjiva roxburghii* Wall has been used in several countries, including Bangladesh, India, Nepal, Sri Lanka, and Indochina. The pharmacological activities of the entire plant are diverse. Different plant components are utilized for different ailments or diseases; for example, leaves and fruits are used for fever and arthritis, and seed oil is used as an antibacterial agent. The plants also exhibit cytotoxic, anti-inflammatory, and antioxidant properties.[3]

MATERIALS AND METHODS:

Plant material: the leaves of plant *Putranjiva roxburghii* Wall were collected from Amarai near Shivaji Stadium Sangli during of November. The plant was identified and authenticated by Mrs. U V. Waghamare Department of Botany Willingdon College, Sangli.

Preparation of extract:

1. Maceration[4]

2. Soxhlet extraction

The collected leaves were separated and washed. They were dried in shade at room temperature. They were crushed and coarsely powdered and sieved through No. 44 mesh sieve. The successive solvent extraction was carried out using Soxhlet apparatus. About 50 gm. of powder of leaves was extracted with 350 ml of ethanol. Aqueous (aq.) extract was prepared by macerating the coarsely powdered leaves in 10% of chloroform water for seven days. The extract was dried in rotary vaccum evaporator. Ethanolic Extract of Leaves *Putranjiva roxburghii* (EELPR) and Aqueous Extract of Leaves *Putranjiva roxburghii* (AELPR) thus obtained were used for phytochemical investigation and for screening in vitro anticancer activity. The in vitro cytotoxicity was assayed by using MTT-(3-[4, 5-dimethylthiazol-2-yl]-2, 5 diphenyl tetrazolium bromide) method.[5, 6]

Preliminary phytochemical:

All above extracts were subjected to phytochemical analysis. They showed presence of flavonoids, alkaloids, saponins, phenolic compound and tannins as major chemical constituents.[7]

In vitro cytotoxic assay by MTT-(3-[4, 5-dimethylthiazol-2-yl]-2, 5 diphenyl tetrazolium bromide) method.

Cell lines: MCF-7 (Michigan Cancer Foundation-7)

Chemicals: Phosphate Buffer Saline, 5 Fluro Uracil, Dimethyl Sulphoxide (DMSO) and MTT reagent

Equipment: Carbon dioxide (CO_2) incubator.

Procedure ethanolic extract: Cells were incubated at a concentration of 1×10^4 cells/ml in culture medium for 24 h at 37°C and 5% CO_2.Cells were seeded at a concentration (70µl) 10^4 cells/well in 100 µl culture medium and 100µl sample PR (10, 40,100 µg/ml) into micro plates respectively (tissue culture grade, and 96 wells). Control wells were incubated with DMSO (0.2% in PBS) and cell line. All samples were incubated in triplicate. Controls were maintained to determine the control cell survival and the percentage of live cells after culture. Cell cultures were incubated for 24 h at 37°C and 5% CO_2 in CO_2 incubator (Thermo scientific BB150) after incubation, the medium was completely removed and Added 20 µl of MTT reagent (5mg/min PBS).After addition of MTT, cells incubated for 4 hrs. at 37ºC in CO2incubator. Observed the wells for formazan crystal formation under microscope. The yellowish MTT was reduced to dark coloured formazan by viable cells only. Registered under Companies Act, 2013 (18 of 2013) - CIN U73100PN2021PTC198266 Registered under CPCSEA 2114/PO/Re/S/20/CPCSEA after removing the medium completely. Added 200µl of DMSO (kept for 10 min) and incubate at 37^0C (wrapped with aluminum foil). Triplicate samples were analyzed by measuring the absorbance of each sample by an Elisa microplate reader (Benesphera E21) at a wavelength of 570 nm.[8]

$$\% \text{ cytotoxicity} = \frac{\text{Reading of control} - \text{Reading of treated cells} \times 100}{\text{Reading of control}}$$

Procedure for aqueous extract:

MCF7 Cells were incubated at a concentration of 1×10^4cells/ml in culture medium for 24 h at 37°C and 5% CO_2.

Cells were seeded at a concentration (100µl) 104cells/well) in 100µl culture medium and 10, 40, 100 µg/ml of Samples into micro plates respectively (tissue culture grade, and 96 wells). Control wells were incubated with DMSO (0.2% in PBS) and cell line. All samples were incubated in triplicate. Controls were maintained to determine the control cell survival and the percentage of live cells after culture. Cell cultures were incubated for 24 h at 37°C and 5% CO_2 in CO_2 incubator. After incubation, the medium was completely removed and Added 20µl of MTT reagent (5mg/ml PBS).

After addition of MTT, cells incubated for 4 hours at 37oC in CO_2 incubator. Observed the wells for formazan crystal formation under microscope. The yellowish MTT was reduced to dark colored formazan by viable cells only. After removing the medium completely. Added 200µlof DMSO (kept for 10 min) and incubate at 370C (wrapped with aluminum foil). Triplicate samples were analyzed by measuring the absorbance of each sample by a micro plate reader at a wavelength of 550 nm. [9, 10, 11, 12]

RESULTS:

Table 1: in vitro anticancer activity of ethanolic extract of *Putranjiva roxburghii* Wall. Against MCF-7 cell by MTT method.

Sr. No.	Sample	Concentration (ug/ml)	OD	Mean	%inhibition	IC_{50} (ug/ml)
1	Control		0.899 0.891 0.837	0.875		
2	Std.5FU	10	0.212 0.201 0.196	0.203	76.08	32.07
		40	0.105 0.117 0.125	0.115	86.85	
		100	0.078 0.093 0.100	0.090	89.71	
3	Ethanolic Sample	10	0.374 0.368 0.354	0.365	58.28	41.10
		40	0.288 0.259 0.354	0.262	70.05	
		100	0.124 0.201 0.114	0.146	83.31	

Table 2: in vitro anticancer activity of aqueous extract of *Putranjiva roxburghii* against MCF-7 cell line by MTT method.

Sr. No.	Sample	(Concentration ug/ml)	OD	Mean	%inhibition	IC_{50} (ug/ml)
1	Control		0.899 0.891 0.837	0.875		
2	Std. 5 FU	10	0.212 0.202 0.196	0.203	76.08	32.15
		40	0.105 0.117 0.196	0.115	86.85	
		100	0.078 0.093 0.100	0.090	89.71	
3	Aqueous Sample	10	0.958 0.956 0.956	0.956	26.85%	30.46
		40	0.811 0.810 0.812	0.811	37.94%	
		100	0.756 0.754 0.756	0.755	42.23%	

DISCUSSION:

Cancer is a leading cause of mortality worldwide and failure of conventional chemotherapy to effect a major reduction in mortality indicates that new approaches were critically needed. Here we have performed in vitro anticancer activity of EELPR and AELPR by MTT assay method. In this method we have used three different concentration 10 ug/ml, 40 ug/ml and 100 ug/ml respectively. Result of table 1 and 2 indicated that all extract tested showed in vitro anticancer activity in concentration depends manner. Ethanolic extract were found to be more potent as compared to aqueous extract.

From the above obtained results of anti-cancer activity i.e. MTT assay. The minimum inhibition concentration of aqueous and ethanolic extract of *Putranjiva roxburghii* Wall.

Table no.1 indicates that most active was ethanolic extract from the leaves with 83.31% cytotoxicity at concentration 100 ug/ml. as compared to std. 5 fluro uracil with 89.71% cytotoxicity. Its IC_{50} was graphically determined and found to be 41.10. Table no.2 indicated that the lowest active was aqueous extract from the leaves with 42.23% cytotoxicity at concentration of 100 ug/ml. as compared to std. 5 fluro uracil with 89.71% cytotoxicity. Its IC_{50} was graphically determined and found to be 30.46

CONCLUSION:

The above result gave a clear view that the plant *Putranjiva roxburghii* Wall is having anticancer potential. Ethanolic extract showed more significant in vitro anticancer activity as compared to aqueous extract. Among the tested extracts, ethanolic extract from the leaves of *Putranjiva roxburghii* Wall exhibited high degree of cytotoxicity. Result obtained by *in vitro* methods. According to result obtained from preliminary phytochemical investigation, all extract have shown presence of flavonoids, alkaloids and polyphenolic compounds. As flavonoids and polyphenolic compounds possess anticancer activity.[13], we can conclude that anticancer activity of Putranjiva roxburghii Wall may be due to presence of flavonoids and polyphenolic compounds.

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