



IN-VITRO ANTICANCER ACTIVITY OF *RUBIA CORDIFOLIA* AGAINST HELA AND HEP-2 CELL LINES

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ABSTRACT

Cancer is the most devastating disease and leading cause of death throughout the world. Natural drugs are under investigation for their selective cytotoxicity to cancer cells. Methanol fraction of *Rubia cordifolia* extract exhibited potent inhibition of Human cervical cancer cell line and Human larynx carcinoma cell line while was found to be less cytotoxic against normal human kidney cells displaying safety for normal cells. *Rubia cordifolia* can be a source of potent pharmacophore for treatment of disease like cancer.

Keywords: *Rubia cordifolia*, Cell line, Anticancer

INTRODUCTION

Rubia cordifolia (Rubiaceae), also known as Indian Madder or Manjisthais is traditionally used as anti-inflammatory, antiseptic and galactopurifier but its anticancer property is not known. Cancer is a dreadful disease and any practical solution in combating this disease is of paramount importance to public health¹. Plants have been used as folk remedies and ethno botanical literature has described the usage of plant extracts. There is an increasing need for search of new compounds with cytotoxic activity as the treatment of cancer with the available anticancer drugs is often unsatisfactory due to the problem cytotoxicity to the normal cells. For the last few decades, phytochemical examination has been making rapid progress and herbal products are becoming popular as sources of possible anticancer compounds².

MATERIALS AND METHODS

Reagents

Trypan blue (Hyclone), Triton X100 (MP Biomedicals), DMSO cell culture grade (MP Biomedicals), Sodium bicarbonate (MP Biomedicals), HYQ® Antibiotic/Antimycotic solution, 100X (10000 U/ml Penicillin G, 10000 µg/ml Streptomycin, 25 µg/ml Amphotericin B) (Hyclone), Penicillin and Streptomycin solution (MP Biomedicals), EDTA (MP Biomedicals), HYQ® DPBS/modified 1X (Dulbecco's phosphate buffer saline without Ca⁺ & Mg⁺) (Hyclone), 0.25% Trypsin 1X (Invitrogen), Cyclophosphamide monohydrate (MP Biomedicals), HBSS -1X (Hank's Balanced Salt solution) (Hyclone), Cell proliferation kit (XTT) 2500 tests (Roche), Ethanol, Methanol, Petroleum ether, Dichloromethane

Media

DMEM (Dulbecco's Modified Eagles medium, low glucose with glutamine) (US Biological), RPMI1640 (with L-glutamine) (Hyclone), FBS (Fetal Bovine Serum, South American origin) (Bioclot), HYQ® SFM HEK-293™ (Hyclone)

Cell lines

HEK 293 (Human Epithelial Kidney cell line), HeLa (Human cervical cancer cell line), HEP-2 (Human larynx carcinoma cell line), all cell lines were purchased from NCCS: National Center for Cell Science, Pune.

Collection and preparation of plant material

The plant sample (roots) of *Rubia cordifolia* was purchased from Yucca enterprise, Mumbai. For taxonomical identification, it was authenticated by Mr. V.R.Patel (Dept. of Pharmacognosy, Baroda college of Pharmacy, Vadodara). After proper identification, the plant samples were cut into small pieces followed by dried and grinded into coarse powder by using high capacity grinding machine

and passed through sieve number 14. It was stored in an airtight container.

Extraction and fractionation procedure

500 g of dried powder of *Rubia cordifolia* was soaked into ethanol and boiled at 80°C for 3 hours to get crude ethanol extracts. The extract was then filtered through cotton followed by Whatman No.1 filter paper and the filtrate thus obtained was concentrated at 40°C with a rotary evaporator (Rotaver). The concentrated extract was dried residue. The yield of the extract was 38.4 g. The crude extract was then dissolved in 10% water in methanol (100 ml) and partitioned between pet-ether (2.8 g), dichloromethane (4.2 g) and methanol fractions (22.9 g)³.

Experimental design

A cytotoxicity property of extracts of roots of *Rubia Cordifolia* was carried out by XTT method against HEK293, HeLa, and HEP-2 cell lines. 2 mg of each plant extract was dissolved in 200 µl of DMSO (dimethyl sulfoxide) then 100 µl of this solution was diluted to 10 ml with DMEM (Dulbecco's Modified Eagles medium, low glucose with glutamine). Thus, final concentration of this stock solution was 100 µg/ml. Then by serial dilution varying concentrations were prepared from the stock solution. Thus the concentrations of the solutions obtained were 100 µg/ml, 33.33 µg/ml, 11.11 µg/ml, 3.70 µg/ml, 1.23 µg/ml, 0.411 µg/ml, 0.137 µg/ml, 0.045 µg/ml, 0.015 µg/ml, 0.005 µg/ml. 2 mg of Cyclophosphamide monohydrate (served as the positive control) was dissolved in 200 µl of DMSO (dimethyl sulfoxide) then 100 µl of this solution was diluted to 10 ml with DMEM (Dulbecco's Modified Eagles medium, low glucose with glutamine). Thus, final concentration of this stock solution was 100 µg/ml. Then by serial dilution varying concentrations were prepared from the stock solution. Thus the concentrations of the solutions obtained were 100 µg/ml, 33.33 µg/ml, 11.11 µg/ml, 3.70 µg/ml, 1.23 µg/ml, 0.411 µg/ml, 0.137 µg/ml, 0.045 µg/ml, 0.015 µg/ml, 0.005 µg/ml. As for negative control 100 µl of DMSO was diluted to 10 ml with DMEM (Dulbecco's Modified Eagles medium, low glucose with glutamine)³.

Cells were preincubated at a concentration of 1×10^6 cells/ml in culture medium for 3 h at 37°C and 5% CO₂. Cells were seeded at a concentration of 5×10^4 cells/well in 100 µl culture medium and various amounts of compound (final concentration e.g. 100 µM - 0.005 µM) into microplates (tissue culture grade, 96 wells, flat bottom). Cell cultures were incubated for 24 h at 37°C and 5% CO₂. 50 µl XTT labeling mixture was added and incubated for 18 h at 37°C and 6.5% CO₂. The spectrophotometrical absorbance of the samples was measured using a microplate (ELISA) reader. The wavelength to measure absorbance of the formazan product was 450 nm according to the filters available for the ELISA reader, used. The reference wavelength was more than 650 nm^{4,5}.

All experiments were performed using three wells for each concentration of each compound tested. The cytotoxicity data was standardized by determining absorbance and calculating the correspondent compound concentrations. Dose response curve was developed for each concentration of each compound tested. IC₅₀ value was determined for each concentration of each compound tested⁶.

RESULTS AND DISCUSSION

In this in vitro cytotoxicity assay, the root extract of *Rubia cordifolia*, exhibited significant cytotoxic activity against HEp-2 cell line with IC₅₀ values of 11.92 µg/ml, 21.44 µg/ml and 29.02 µg/ml for methanol fraction, pet-ether fraction and dichloromethane fraction respectively, where good cytotoxicity were shown against HeLa cell line with IC₅₀ values of 23.12 µg/ml, 38.13 µg/ml, 48.87 µg/ml for methanol fraction, pet-ether fraction and dichloromethane fraction respectively. None of the fraction of the extract was found to be cytotoxic against HEK293 cell line in the concentration range of 0.05

µg/ml to 100 µg/ml. The IC₅₀ values are given in table 1. Graphical representation is shown in figure 1.

Table 1: IC₅₀ values (µg/ml) of standard Cyclophosphamide monohydrate and three different extracts of *Rubia cordifolia* (Rubiaceae) against HEK293, HEp-2 and HeLa cell lines.

Sample	IC ₅₀ VALUES (µg/ml)		
	CELL LINES USED		
	HEK293	HeLa	HEp-2
Cyclophosphamide monohydrate*	>100	3.63	4.81
Dichloromethane fraction	>100	48.87	29.02
Methanol fraction	>100	23.12	11.92
Pet-ether fraction	>100	38.13	21.44

*(Positive control)

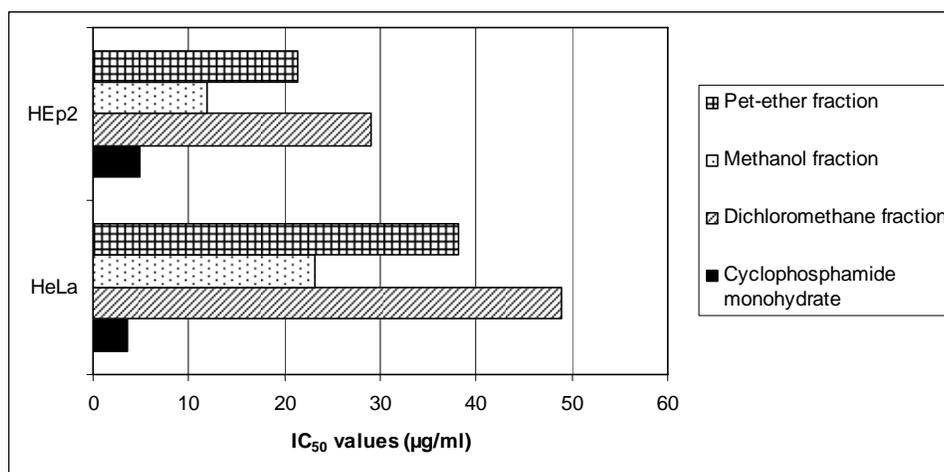


Fig.1: Graphical representation of IC₅₀ values (µg/ml) of standard Cyclophosphamide monohydrate and three different extracts of *Rubia cordifolia* (Rubiaceae) against HEp-2 and HeLa cell lines.

CONCLUSION

Study results (Table 1) show that root extracts of *Rubia Cordifolia* is promisingly cytotoxic against human larynx carcinoma and human cervical cancer. None of the fraction of the extract was found to be cytotoxic against the normal cell line (HEK293) in the given range of concentration.

So, this plant extracts may have clinical and therapeutic proposition in the most life threaten disease like cancer and further studies are required to investigate these plant samples as antineoplastic agents.

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