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Potent antitumor activity of *Rubia cordifolia*

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Abstract

Background: Cancer is a leading cause of death. *Rubia cordifolia* is a traditional ayurvedic medicine being used as a remedy for various ailments. **Results:** Dichloromethane fraction of *Rubia cordifolia* extract exhibited potent inhibition of human leukaemia cell line and human histolytic lymphoma cell line while was found to be lesser active against normal human kidney cells displaying safety for normal cells. **Conclusion:** *Rubia cordifolia* can be a source of potent pharmacophore for treatment of disease like cancer.

Keywords: Rubia cordifolia; Cell line; Anticancer

Introduction

Cancer is a dreadful disease and any practical solution in combating this disease is of paramount importance to public health [6]. Plants have been used as folk remedies and ethnobotanical literature has described the usage of plant extracts [7]. 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 [7]. For the last few decades, phytochemical examination has been making rapid progress and herbal products are becoming popular as sources of plausible anticancer compounds [7].

Rubia cordifolia (Rubiaceae), also known as Indian Madder or Manjistha is traditionally used as anti-inflammatory, antiseptic and galactopurifier but its anticancer property is yet not known.

Material 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,10000µg/ml Streptomycin, 25 µg/ml Amphotericin B) (Hyclone), Penicillin and Streptomycin solution (MP Biomedicals), EDTA (MP Biomedicals), HYQ® DPBS/modified 1X (Dulbecoo'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 (Dulbecoos Modified Eagels medium, low glucose with glutamine) (US Biological), RPMI1640 (with L-glutamine) (Hyclone,), FBS (Fetal Bovine Serum, South American origin)

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(Bioclot), HYQ® SFM HEK-293^{TM} (Hyclone).

Cell lines

HEK 293 (Human Epithelial Kidney cell line), U937 (Human Histolytic lymphoma cell line), HL 60 (Human Myeloid leukemia 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 80ºC 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 40ºC 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) [5].

Experimental design

A cytotoxicity property of extracts of roots of *Rubia Cordifolia* was carried out by XTT method against HEK293, HL60, and U937 cell lines. 2 mg of each plant extract was dissolved in 200µl of DMSO (dimethyl sulfoxide) then 100µl of this solution was diluted to 10ml with DMEM (Dulbecoos Modified Eagels medium, low glucose with glutamine). Thus, final concentration of this stock solution was 100µg/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 200µl of DMSO (dimethyl sulfoxide) then 100µl of this solution was diluted to 10ml with DMEM (Dulbecoos Modified Eagels medium, low glucose with glutamine). Thus, final concentration of this stock solution was 100µg/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 100µl of DMSO was diluted to 10 ml with DMEM (Dulbecoos Modified Eagels medium, low glucose with glutamine) [5].

Cells were preincubated at a concentration of 1×10^6 cells/ml in culture medium for 3 h at 37°C and 5% CO_2. 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. 100µM - 0.005µM) into microplates (tissue culture grade, 96 wells, flat bottom). Cell cultures were incubated for 24 h at 37°C and 5% CO_2. 50 µl XTT labeling mixture was added and incubated for 18 h at 37°C and 6.5% CO_2. 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 [2-4].

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. IC_{50}

value was determined for each concentration of each compound tested [1].

Table.1 IC_{50} values (µg/ml) of standard Cyclophosphamide monohydrate and three different extracts of *Rubia cordifolia* (Rubiaceae) against HEK 293, U937 and HL60 cell lines.

Sample	IC_{50} Values (µg/ml) Cell lines used		
	HEK293	HL 60	U937
Cyclophosphamide monohydrate*	>100	0.543	0.781
Dichloromethane fraction	>100	16.72	41.59
Methanol fraction	>100	8.57	27.23
Pet-ether fraction	>100	10.51	35.44

*(Positive control)

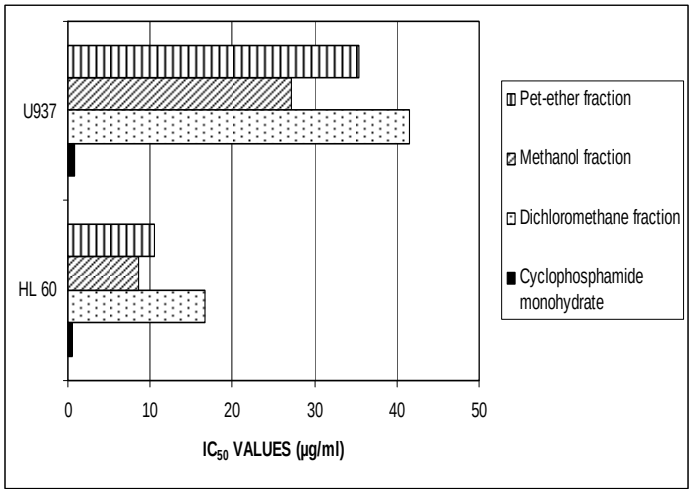

Figure 1. Graphical representation of IC_{50} values (µg/ml) of standard Cyclophosphamide monohydrate and three different extracts of *Rubia cordifolia* (Rubiaceae) against HEK 293, U937 and HL60 cell lines.

Result and discussion

In this in vitro cytotoxicity assay, the root extract of *Rubia cordifolia*, exhibited significant cytotoxic activity against HL60 cell line with IC_{50} values of 8.57 µg/ml, 10.51 µg/ml and 16.72 µg/ml for methanol fraction, pet-ether fraction and dichloromethane fraction respectively, where moderate cytotoxicity was shown against U937 cell line with IC50 values of 27.33 µg/ml, 35.44 µg/ml, 41.59 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 0.05-100 µg/ml.

Conclusion

Study results (Table 1) show that root extracts of *Rubia Cordifolia* is promisingly cytotoxic and they might have antitumor activity against myeloid leukemia and Histolytic lymphoma. 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 this plant as source of antineoplastic agents.

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