



Evaluation of the *In Vitro* Cytotoxic Activity of *Jania rubens* Against Jurkat and Molt-4 Human Cancer Cell Lines

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ABSTRACT

Cancer is a malignant disease that is characterized by rapid and uncontrolled proliferation of abnormal cells which may accumulate together to form a tumour mass or diffuse throughout the body. It is second to heart disease among the leading noncommunicable diseases and is a major threat to mankind. The present study aims at the preliminary phytochemical screening and cytotoxic evaluation of methanol extract of *Jania rubens* against Jurkat and molt-4 human cancer cell lines. The algae *Jania rubens* Linn was collected manually from East Coast of Bay of Bengal. Different concentrations (0, 1.953, 3.908, 7.8125, 15.625, 31.25, 62.5, 125, 250, 500 and 100 µg/mL) of the methanol extract from *Jania rubens* were prepared and tested for its potential cytotoxic activity against Jurkat and molt-4 human lymphoblastic leukaemic cell lines. The cells were treated with different concentrations of the algal extract and the cytotoxicity of the extract was evaluated by methyl thiazolyl tetrazolium (MTT) assay. Also, the number of viable cells was determined by trypan blue test. IC₅₀ values of the algal extract against the cell lines were found to be 62.5 and 60.25 µg/mL, respectively. The methanol crude extract of the algae *Jania rubens* exhibited moderate cytotoxic activity against Jurkat and Molt-4 cell lines and may be a good source of anticancer agents against leukaemia.

Keywords: *Jania rubens*, cytotoxic, Jurkat, molt-4, methyl thiazolyl tetrazolium (MTT) assay.

Introduction

Cancer is a dreadful disease, killing around 7 million people every year.¹ More than 80% of cancer-related deaths are due to carcinoma of the lung, breast, prostate, colorectal, and pancreas.² Lung and colorectal cancers are responsible for the first and third most cancer-related deaths in men and women. Breast cancer in women and prostate cancer in men rank second.³ Though Chemotherapy is considered as the standard method of treatment, it is often associated with severe toxicity and huge cost. Naturally obtained compounds are considered safer and easily biodegradable than synthetic compounds and the problem of drug resistance observed in synthetic drugs can also be reduced. Nowadays, most of the research work has been focused on deriving anticancer agents from natural sources such as seaweeds, plants, etc.⁴ Marine algae are one of the natural resources in the marine ecosystem. They contain various biologically active compounds which have been used as a source of food and medicine. Until now, more than 2400 marine natural products have been isolated from seaweeds of subtropical and tropical populations.⁵ During the last decade, about 2500 new metabolites with antiproliferative activity have been reported, a recent review discussed 68 new marine-derived anticancer chemical entities, most of them with undetermined modes of action.⁶ Recent findings showed that seaweeds also contained

antiviral, antibacterial, antifungal and antitumour potentials, among numerous others.⁷⁻¹⁰

Here, in our present study we evaluated the *in vitro* cytotoxic activity of *Jania rubens*, a red alga which is found in many parts of the world such as Mediterranean, Black Sea, North-Eastern Atlantic (from Norway to Morocco), Indian Ocean and the China Sea, and has been reported to possess many biological activities such as antibacterial, antifungal, anthelmintic, anticancer, analgesic, antipyretic and anti-inflammatory activities.^{11, 12} We have tested the methanol extract of the red algae for its potential cytotoxic activity against Jurkat and molt-4 cells which are two kinds of human leukemic cell lines.

Materials and Methods

Collection and Identification of Plant material

The algae *Jania rubens* Linn was collected manually from East Coast of Bay of Bengal during the month of May 2017, it was identified and authenticated by Dr. Nirmala of the Department of Botany, Osmania University, Telangana, India. A Voucher specimen (PH- 805) was deposited in the herbarium of the college. The biomass was cleaned with double distilled water several times to neutralize the pH, dried in hot air oven at 75°C for 24 h and weighed. The dried algae were powdered and passed through #200 sieve.

Preparation of the extract

The methanol extract of the algae was prepared by treating 1.97 kg of the dried algal mass with methanol for 24 h using a soxhlet apparatus.¹³ The resulting extract was concentrated and dried under reduced pressure at 40–45°C using a rotary evaporator and were preserved at 4°C.

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Phytochemical screening of methanol extract

The preliminary phytochemical screening of methanol extract of *Jania rubens* was performed to detect the presence of alkaloids, glycosides, flavonoids, lipids, phenolic compounds, steroids and terpenoids.¹⁴

Cell lines

Human Jurkat (lymphoblast-like) and molt-4 (lymphoblast-like) cell lines were selected as representatives of human leukemic cell lines which were gift samples received from Tata Memorial Institute of Cancer Research, Mumbai, India. Cells were maintained in RPMI-1640 medium in CO₂ incubator at 37°C with 98% humidity and 5% CO₂ gas environment.

Trypan blue exclusion test

Viable count of the cancer cells before and after treatment with the algal extract was done by using trypan blue test. It is based on the principle that the chromophore of the dye is negatively charged and can interact with the cell only if the membrane is damaged.¹⁵ Hence in the present test, all the cells that exclude the dye are viable. The test was conducted by treating cancer cells (2×10^4 cells/well) with 0, 1.953, 3.908, 7.8125, 15.625, 31.25, 62.5, 125, 250, 500 and 100 µg/mL of the algal extract for 72 h at 37°C in the presence of 5% CO₂. After incubation for 72 h, 20 µL of the medium and equal volume of trypan blue were mixed and viable and dead cells were enumerated using a Neubauer counting chamber.¹⁶

$$\% \text{ Dead cells} = \frac{\text{No. of dead cells}}{\text{Sum of the live cells and dead cells}} \times 100$$

Methyl thiazolyl tetrazolium (MTT) assay test

The cytotoxicity of the algal extract was evaluated using MTT assay. This assay depends upon the principle that the yellow coloured compound (MTT [3-(4, 5-dimethyl thiazole-2-yl)-2, 5-diphenyl tetrazolium bromide]) gets converted to a blue coloured compound by the action of mitochondrial enzymes which are only present in the viable cells. Hence the intensity of the colour produced is directly proportional to the number of viable cells. In this method, 10 µL of MTT stock solution (5 mg/mL in PBS) was incorporated into wells containing 90 µL medium and were exposed to different concentrations (0, 1.953, 3.908, 7.8125, 15.625, 31.25, 62.5, 125, 250, 500 and 100 µg/mL prepared in DMSO) of the algal extract for 72 h. The microplate was incubated at 37°C for 4 h and then, the optical density of each well was read by microplate reader at 540 nm.¹⁷

$$\% \text{ Cell Viability} = \frac{[\text{O.D. of control} - \text{O.D. of test compound}]}{\text{O.D. of control}} \times 100$$

(O.D = Optical density).

Results and Discussion

The results of trypan blue exclusion test are presented in the Figures 1 and 2. Data obtained in the present test revealed that the number of Jurkat cells in the negative control wells (without extract) increased from 2×10^4 to 7×10^4 after 72 h of incubation. In the extract treated wells, the number of viable cells decreased in a concentration-dependent manner which demonstrated the cytotoxic activity of the algal extract on Jurkat cells. Dead cells were identified in all microplate wells. The highest numbers of dead cells were present in the well containing a higher concentration of the algal extract. The results of the cytotoxicity of the algal extract against Jurkat cell line using the MTT assay is shown in Figure 3 with IC₅₀ of 62.5 µg/mL.

The results of the trypan blue exclusion tests for viable and dead cells after exposure to different concentrations of the extract against molt-4 cells is presented in Figure 2. As shown in the Figure 2, the number of viable molt-4 cells in the negative control (without extract) well increased from 2×10^4 to 6.8×10^4 after 72 h of incubation. The extract showed significant concentration-dependent inhibition of molt-4 cells and it was found that the extract is cytotoxic and produced 50% of cell death at a concentration of 60.25 µg/mL against molt-4 cell lines which was further confirmed by the results of the MTT assay (Figure 4).

The red algae *Jania rubens* belonging to the family Corallinaceae, Rhodophyta, has been reported to possess several pharmacological activities. The aqueous extract of *J. rubens* has been shown to possess antibacterial and antifungal activities^{18,19} while the dichloromethane

Table 1: Preliminary phytochemical screening of methanol extracts.

Chemical tests	Results
Alkaloids	+
Triterpenoids	+
Steroids	+
Tannin	+
Saponin	-
Coumarins	+
Terpenoids	+
Quinine	+
Phytosteroids	+
Flavonoids	+
Phlobtannins	+

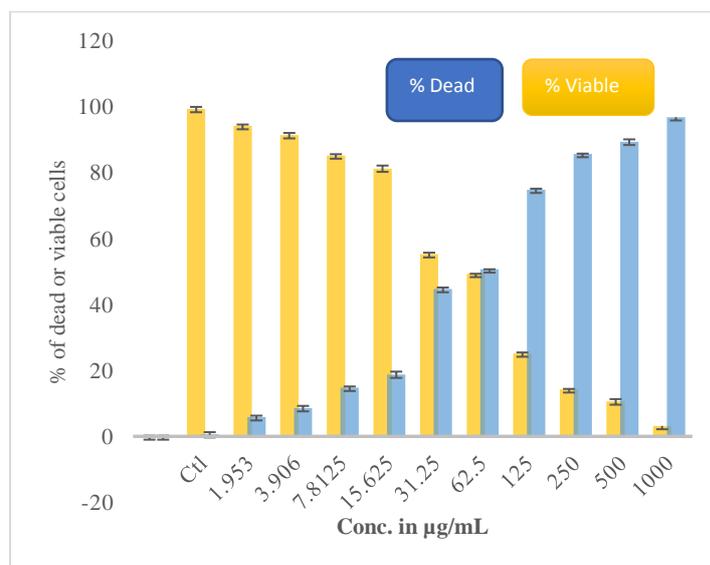


Figure 1: Result of trypan blue exclusion test on Jurkat cell line 72 h post treatment with *J. rubens* extract.

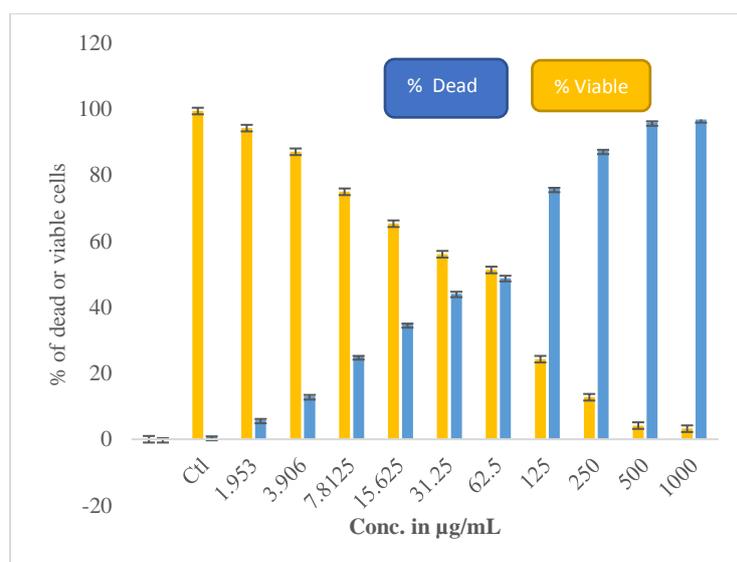


Figure 2: Result of trypan blue exclusion test on molt-4 cell line 72 h post treatment with *J. rubens* extract.

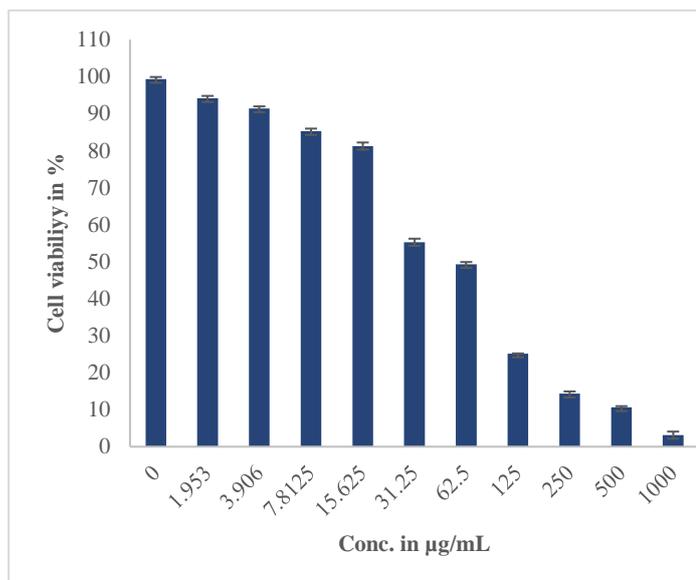


Figure 3: Percentage cell viability of Jurkat cell line after treatment with extract in the MTT assay.

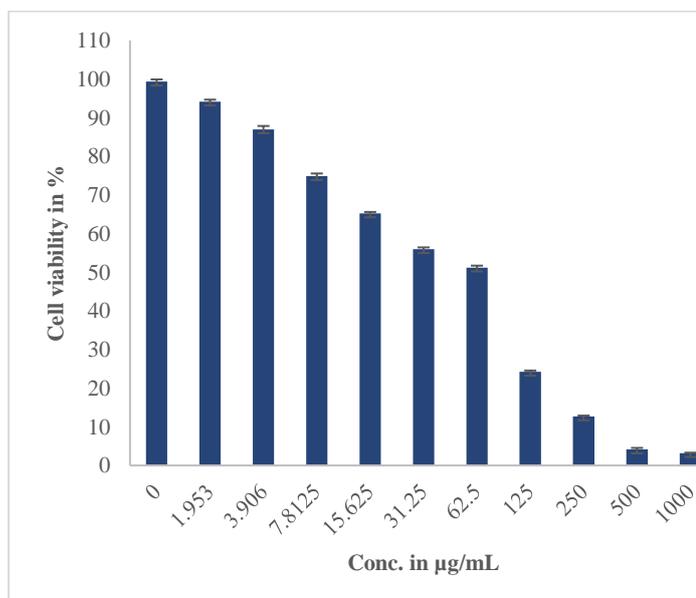


Figure 4: Percentage cell viability of molt-4 cell line after treatment with extract in the MTT assay.

extract was found to possess cytotoxic activity against the KB tumour cell line (human buccal epidermal carcinoma).^{20,21} *J. rubens* collected from the Egyptian seashore was reported to exhibit significant analgesic, antipyretic and antiinflammatory activities.²² In the study conducted by Salunke Mohini *et al.* on the methanol extracts of *Jania rubens* in ascetic tumour bearing mouse, significant reduction in the growth of tumour was observed.²³

In the present study, the methanol extract of *Jania rubens* was investigated for its cytotoxic activity against Jurkat and molt-4 cell lines. The methanol extract of *Jania rubens* exhibited moderate cytotoxic activity against both cancer cell lines. Phytochemical screening showed the presence of various phytoconstituents such as sterols, alkaloids, triterpenoids, flavonoids and tannins (Table 1). The cytotoxic activity of the extract may be due to the presence of these phytoconstituents. The present study has shown the cytotoxic activity of *Jania rubens* against Jurkat and Molt-4 human cancer cell lines and may be a promising source of chemotherapeutic agent(s) against leukaemia.

Conclusion

The methanol extract of *Jania rubens* possesses moderate cytotoxic activity against Jurkat and Molt-4 human cancer cell lines. Further studies may be required to determine the possible mechanism of action and safety levels of the extract.

Conflict of interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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