



RESEARCH ARTICLE

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A Fission Yeast Bioassay for Rapid Screening for Antiproliferative Agents from *Bauhinia variegata* and *Leptadenia reticulata*

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ABSTRACT

The aim of the present study was to evaluate the antiproliferative activity of crude extracts from leaves of *Bauhinia variegata* and *Leptadenia reticulata* using fission yeast *Schizosaccharomyces pombe* as a model. The crude extracts of both plants were prepared by three experimental conditions (25°C, elevated temperature and ultrasound waves) and four solvent systems [Water, Methanol, Water: Methanol (1:1) and Chloroform: Methanol (1:1)]. MTT assay was performed for estimating the cell viability and the results were expressed as % growth inhibition. Overall based on the MTT assay performed on yeast cells, Water: Methanol, Methanol and Water extracts showed higher antiproliferative activity than the positive control (Paclitaxel) in a dose-dependent manner. For *Bauhinia variegata* and *Leptadenia reticulata*, methanol extract (25°C temperature & elevated temperature, respectively) exhibited highest inhibition which was approximately 80%. In conclusion, this study has indicated that *Bauhinia variegata* and *Leptadenia reticulata* extracts possess strong antiproliferative activity. Furthermore, it could act as a potential source for identifying new molecules with chemotherapeutic potential against cancer.

Keywords: Medicinal plants, Fission yeast, *S. pombe*, Antiproliferative activity, MTT Assay.

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INTRODUCTION

As per World Health Organization, around 70-95% of the population in developing countries rely upon drugs derived from medicinal plants to meet their primary healthcare needs. [1-2] Medicinal plants have played an important role in pharmacological research and drug development as they are known to provide bioactive

compounds as well as lead materials for synthesis of new drugs. [3] More importantly, medicinal plants have made significant contribution in the discovery and development of anticancer agents. Some of the well-known examples of medicinal plant derived anticancer drugs are Camptothecin, Podophyllotoxin and Paclitaxel. [4] Therefore, exploring the potential of

different medicinal plants as a source of new anticancer agents based on their traditional uses could lead to discovery of new molecules.

Bauhinia variegata Linn (Caesalpiniaceae) has been used in treating bronchitis, leprosy, tumors and ulcer. [5-6] Also, chemopreventive, anticarcinogenic and antimutagenic potentials of the plant has been reported. [7-8] *Leptadenia reticulata* Wight & Arn (Asclepiadaceae) possesses various pharmacological activities like antibacterial [9], antifungal [10], analgesic, anti-inflammatory, anti-lipoxygenase. [11] Also, anticarcinogenic potential of the ethanolic extracts have been reported against Dalton's Ascitic Lymphoma. [12]

In the present study, yeast was used as a model organism as it has significant similarities to mammalian cells at the molecular and organelle level. [13] It has relatively short doubling time and thus allows examining exposure to drugs over many population doublings. [14] Fission yeast has served as an excellent model organism for studying processes like cell-cycle control, DNA repair and recombination, mitosis and meiosis, and the checkpoint controls important for genome stability. [15] It is widely known that pathways related to cancer-related and genes corresponding to those pathways are conserved between yeast and humans. [16] As a tool for anticancer drug screening, fission yeast *Schizosaccharomyces pombe* has gained acceptance because of extensive conservation of cellular processes between yeast and mammalian cells. [14] *S. pombe* as a model offers several advantages such as: a) it has a cell division pattern similar to mammalian cells [17]; b) basic mechanisms such as DNA metabolism and cell proliferation control are conserved between yeast and human cells [18]; c) biological patterns that are comparable to human cancer cells. [17] Hence, our study used fission yeast mutant having an altered cell cycle to perform a pilot screen on a range of extracts prepared from the plant. Because of its altered cell cycle, this mutant has rapid proliferation rate and hence it mimics a cancer cell.

MATERIALS AND METHODS

The leaves of *Bauhinia variegata* and *Leptadenia reticulata* was collected from Xavier Residence c/o St. Xavier's College Campus, Ahmedabad. The plant was authenticated by Botanist Dr. Hitesh Solanki from Botany Department of Gujarat University. Leaves were thoroughly washed with running tap water to remove the dirt particles followed by distilled water and were then shade dried. The dried leaf was then crushed into fine powder and subjected to extraction. The crude extracts were prepared by using three experimental conditions and four solvent systems. The purpose of using different methods and solvents was to ensure effective extraction of phytochemicals from plant material, generation of range of phytochemicals and identifying best combination of experimental conditions and solvent for extraction. The powder to

solvent (w/v) ratio was kept 1:10. [19] The elevated temperature extraction was carried out in a hot water bath below the boiling point of the respective solvents used for extraction. The temperature used for each solvent system was: (i) Water (90°C); ii) Methanol (55°C); iii) Water: Methanol (65°C); iv) Chloroform: Methanol (50°C). Sonication was performed using ultra sound waves with aid of bath sonicator. [20]

For elevated temperature and sonication extraction, one gram powder was taken in stoppered tube and 10 ml of solvent was added to it which was then incubated in a hot water bath or bath sonicator for one hour. At the end of the incubation period, the filtrate was collected in a pre-weighed beaker. The extraction was performed in this manner until the solvent became colourless. In case of cold extraction (i.e. 25°C temperature), the solvents were changed every 24 hours and constant agitation was provided at 100 rpm. [21] The extracts were filtered using Whatman no. 1 filter paper. The collected filtrate was pooled and evaporated to dryness until a constant weight was achieved. Then stocks of the extract at concentration of 100 mg/ml were prepared in DMSO and stored at -20°C until further use.

Determination of antiproliferative activity using *Schizosaccharomyces pombe*

Schizosaccharomyces pombe was procured from the National Collection of Yeast Cultures (Accession No. NCYC 1683). Yeast cells were cultured in YPD medium (1% Yeast extract, 2% Peptone and 2% Dextrose) and incubated overnight at 25°C. The antiproliferative activity of the extracts was measured by 3-(4, 5-dimethylthiazol-2-yl) 2, 5- diphenyltetrazolium bromide (MTT) assay. Briefly, the optical density (OD) of an overnight incubated culture was read at 600 nm (0.1 OD equates to $\sim 1.5\text{--}2 \times 10^6$ cells/mL) to measure culture biomass. [22] The culture was diluted to achieve desired cell concentration and the cells (4000 cells/well) were seeded to a 96 well plate. The cells were treated with different concentrations of the extracts (250, 500 and 1000 µg/ml) from stocks and incubated for 24 hours. Paclitaxel was used as a positive control in the experiment. [23] DMSO was used as a vehicle control and untreated cells were taken as negative control. Following the incubation period, MTT dye (5 mg/ml) was added to the wells and incubated for four hours in the dark. The formazan crystals formed at the end of the incubation period were dissolved in DMSO. The absorbance was read at 570 nm using Multiskan Go microplate reader. The percentage cell viability was calculated using the following formula

$$\% \text{ Cell Viability} = \frac{[(\text{OD}_{\text{sample}} - \text{OD}_{\text{blank}})]}{(\text{OD}_{\text{control}} - \text{OD}_{\text{blank}})} \times 100 \quad (1)$$

Statistical Analysis

The results were expressed in terms of percentage growth inhibition. Non-linear regression analysis was used to calculate the IC₅₀ values. All the experiments were performed in triplicates and the results are shown below as Mean $\pm$ Standard deviation.

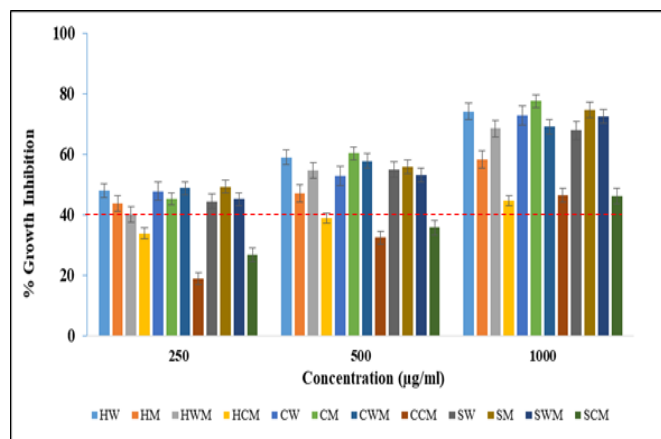


Fig. 1: Growth inhibitory effect of *Bauhinia variegata* Linn crude extracts on *S. pombe* (NCYC 1683)

The cells were treated with different concentration of extracts for 24 hrs. The % growth inhibition was represented as Mean $\pm$ SD (n=3). The crude extracts were prepared at Elevated temperature (H), 25°C (C) and Sonication (S) method. The solvent system used was Water (W); Methanol (M); Water: Methanol (WM); and Chloroform: Methanol (CM). In the graph abbreviation used represents method (H, C and S) and solvent system (W, M, WM and CM). The dotted line represents cell growth inhibition (40%) by positive control Paclitaxel.

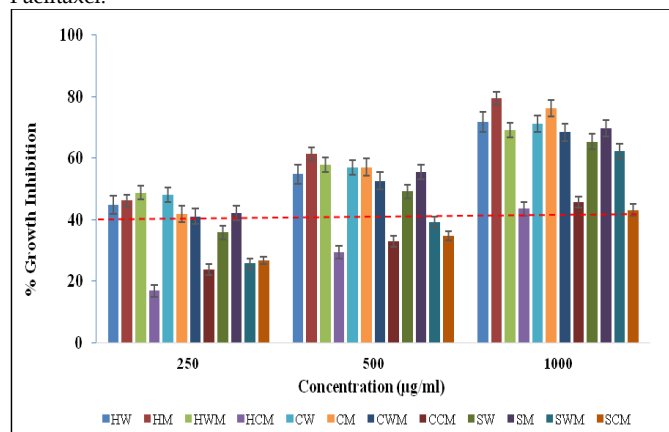


Fig. 2: Growth inhibitory effect of *Leptadenia reticulata* Wight & Arn crude extracts on *S. pombe* (NCYC 1683)

The cells were treated with different concentration of extracts for 24 hrs. The % growth inhibition was represented as Mean $\pm$ SD (n=3). The crude extracts were prepared at Elevated temperature (H), 25°C (C) and Sonication (S) method. The solvent system used were Water (W); Methanol (M); Water: Methanol (WM); and Chloroform: Methanol (CM). In the graph abbreviation used represents method (H, C and S) and solvent system (W, M, WM and CM). The dotted line represents cell growth inhibition (40%) by positive control Paclitaxel.

RESULTS

For evaluating the antiproliferative activity of crude extracts using *S. pombe*, fresh culture having optical density 0.8 was used for the experiment. The crude extracts of *Bauhinia variegata* Linn and *Leptadenia reticulata* (Retz.) Wight & Arn were analysed for decrease in cell viability using the MTT assay in mutant yeast. Cell viability can be correlated to antiproliferative effect of the extract. If the extract is cytotoxic it will decrease the number of viable cells compared to that in the control. All the 24 extracts (12 extracts per plant) were screened at concentrations of 250, 500 and 1000 µg/ml on *S. pombe* (NCYC 1683). Figure 1 & 2 represent the percentage cell growth

inhibition of *S. pombe* treated with crude extracts of all three plants at different doses. It was clearly observed that the decrease in the growth of yeast cells was dose dependent. Moreover, the growth inhibition capacity of the extracts was compared with that of the standard drug Paclitaxel used in the treatment of cancer. When cells were treated with Paclitaxel at 2.5 nm concentration the percentage growth inhibition of yeast cells was approximately 40%. Therefore, in our study, we have chosen 40% cell growth inhibition as a screening criteria for crude extracts. The crude extracts showing inhibition higher than 40% were considered to have potential cytotoxic activity and were selected for secondary screening on cancer cell lines.

For *Bauhinia variegata* (Fig. 1), the antiproliferative activity of extracts prepared at elevated temperature ranged from 33% to 74% after incubation of 24 hours. The antiproliferative activity of extracts prepared at 25°C temperature ranged from 18% to 78%. Next, the antiproliferative activity of extracts prepared at sonication ranged from 26% to 75%. Among the four solvent systems used for extraction, it was observed that the water extracts showed highest growth inhibition whereas chloroform: methanol extracts showed lowest inhibition. Out of the three treatment methods used for extraction, 25°C temperature showed highest inhibition followed by elevated extraction and lowest was observed in sonication extraction.

For *Leptadenia reticulata* (Fig. 2), the antiproliferative activity of extracts prepared at elevated temperature ranged from 16% to 79% after incubation of 24 hours. The antiproliferative activity of extracts prepared at 25°C temperature ranged from 23% to 76%. Next, the antiproliferative activity of extracts prepared at sonication ranged from 25% to 70%. Among the four solvent systems used for extraction, it was observed that the methanol extracts showed highest growth inhibition whereas chloroform: methanol extracts showed lowest inhibition. Out of the three treatment methods used for extraction, elevated temperature extraction showed highest inhibition followed by 25°C and lowest was observed in sonication extraction.

DISCUSSION

In the present study, as a preliminary screening the crude extracts were evaluated for its antiproliferative activity using a mutant fission yeast *Schizosaccharomyces pombe* strain using 1-(4,5-dimethylthiazol-2-yl)-3,5-diphenyltetrazolium bromide (MTT) assay. [24] This mutant yeast has modified cdc gene, and thus, has an altered cell cycle. [18] An altered cell cycle gene would cause a loss in cell cycle regulation which is often the situation in any cancer cell. Thus, by utilizing this mutant yeast one can identify crude extracts with potential antiproliferative activity from the range of extracts of the three plants prepared in our study. Similar studies have been reported where mutant yeast was used as screening tool for identifying bioactive molecules. In a study, a bioactive compound known as

2', 4' - dihydroxychalcone from leaves of *Corema album* was identified using *S. pombe*.^[18] Additionally, the mechanism of action of a known phytochemical Plumbagin (a well-known plant derived anticancer compound) was elucidated using *S. pombe*.^[17] Furthermore, cytotoxic effect of various extracts from *Phyllanthus emblica* L.^[25] and *Nyctanthes arborescens* L.^[26] were tested using *S. pombe*. Based on these studies, we tested the antiproliferative potential of our crude extracts on *S. pombe*.

From this screening, we identified that for *Bauhinia variegata* Linn and *Leptadenia reticulata* the water, methanol, and water: methanol extracts of all three methods showed higher growth inhibition as compared to Paclitaxel (a chemotherapeutic drug used for cancer treatment). The difference in the antiproliferative effects between different extracts may have resulted from the different phytoconstituents and their concentrations contained in the extracts due to the sensitivity to the solvent used and mode of extraction. On the basis of this pre-screen extracts with potential cytotoxic activity can be selected for secondary screening against panel of cancer cell lines.

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