

ANTICANCER ACTIVITY OF VARIOUS EXTRACTS OF *MUSA ROSACEA*, *AVICENNIA MARINA* AND *BOMBEX CEIBA*

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ABSTRACT

Objective: In the present study, the anticancer activity of the various extracts of *M. rosacea*, *A. marina* and *B. ceiba* was evaluated.

Method: Different extracts of *M. rosacea*, *A. marina* and *B. ceiba* on human leukemia cell lines using SRB assay using HL60 cell lines with appropriate positive control (Adriamycin).

Result: The ethyl acetate extract of *M. rosacea* exhibited more activity against HL60 cell lines but the inhibitory effect of this was observed to be a little bit weaker than that shown by Adriamycin and more activity compared to *A. marina* and *B. ceiba* plants extracts.

Conclusion: The results support the folkloric usage of the studied plant and confirmed that the studied plant possesses the constituents with cytotoxic properties that can be useful for developing anticancer agents.

Keywords: *Musa rosacea*, *Avicennia marina*, *Bombax ceiba*, Anticancer activity and HL60 cell lines.

INTRODUCTION

Cancer is the loss of control over the mechanisms that governs "cell survival, proliferation and differentiation" of cell which leads to qualitative and quantitative chromosomal abnormalities (genetically instability). Cells undergo neoplastic transformation to "tumor cells" [1].

In spite of good advancements for diagnosis and treatment, cancer is still a big threat to our society [2]. This is the second most common disease after cardiovascular disorders for maximum deaths in the world. It accounts for about 23 and 7% deaths in USA and India, respectively. The world's population is expected to be 7.5 billion by 2020 and approximations predict that about 15.0 million new cancer cases will be diagnosed; with deaths of about 12.0 million cancer patients [3]. It is believed that in near future the number of cancer patients will increase in the developing and under developed countries, which may rise up to 70%; a serious issue for all of us. The magnitude of cancer problem in the Indian Sub-continent (sheer numbers) is increasing due to poor to moderate living standards [4] and inadequate medical facilities. Most frequently observed cancers in Indian population are of lungs, breast, colon, rectum, stomach and liver. The medicinal properties of plants have been investigated in the recent scientific developments throughout the world, due to their potent against several disease, no side effects and economic viability. Several compounds widely distributed in plants which have been reported to exert multiple biological effect, including antioxidant, free radical scavenging abilities, anti-inflammatory, anti-carcinogenic etc [5-8]. Therefore, it is important to study the status of cancers in India so that advance measures may be taken to control this havoc in near future. In view of these facts, attempts have been made to study the status of cancers in India including its causes, preventive measures, effect on Indian economy and comparison with global scenario.

According to folkloric use, *Musa rosacea*, *Avicennia marina* and *Bombax ceiba* plants were used for the abortion by different tribal communities of Andhra Pradesh [9-12]. So, the present study, we tested different extracts of *M. rosacea*, *A. marina* and *B. ceiba* on human leukemia cell lines (HL60 cell lines) using SRB assay.

MATERIALS AND METHODS**Plant materials and Preparation of extracts**

Musa rosacea whole plant, *Avicennia marina* leaves and *Bombax ceiba* seeds were collected at Araku valley and Visakhapatnam, Andhra Pradesh. The plants were authenticated by taxonomist (Prof.

M. Venkaiah, Department of Botany, Andhra University. The collected plant material was shade dried and pulverized into powder. The powdered material was used for extraction with different solvents (Hexane, Ethyl Acetate, Methanol and Ethanol (70%)) using maceration process. Then the some extracts were used for screening anticancer activity.

Chemicals

Test drugs: Different extracts of *M. rosacea*, *A. marina* and *B. ceiba* (at 12, 20, 40 and 80 µg/ml doses)

Standard drug: Adriamycin

Cell lines: human leukemia cell lines (HL60 cell lines)

SRB assay [13, 14]

The monolayer cell culture was trypsinized and the cell culture was adjusted to 5×10^3 cell/ml. To each well of the 96 well micro tray plates, 0.1ml of the diluted cell suspension was added. After 24hrs, when a partial monolayer had formed the supernatant was flicked off, the monolayer was washed once with medium and 100 µl of different concentration of extract were added to the cells in micro tray plates. The plates were then incubated at 37°C for 3 days in 5% CO₂ atmosphere, microscopic examination was carried out and observations were recorded every 24 hr. after 72 hrs, 25µl of 50% TCA was gently added to the wells in such a way that it formed a thin-layer over the extract. The plates were incubated at 4°C for 1hr. They were flicked and washed 5 times with water to remove traces of the medium, extract and serum and air dried. They were stained with SRB (0.4% prepared in 1% acetic acid) for 30 mins. The unbound dye was then removed by rapidly washing 4 times with 1% acetic acid. The plates were then air dried. Tris base (10mM, 100µl) was then added to the wells to solubilize the dye. The plates were shaken vigorously for 5 min. The absorbance was measured using a micro plate reader at a wavelength of 540nm. The percentage control growth, GI50 (conc. of drug or test extract needed to inhibit cell growth by 50%), TGI and LC50 were calculated from dose response curves for each cell line.

RESULTS AND DISCUSSION

A significant part of drug discovery in the last years has been focused on agents to prevent or treat many diseases including cancer. In developing countries, cancer is amongst the three most common causes of death and morbidity. Treatment for cancer involves surgery, radiotherapy and chemotherapy and often a combination of two or all three is employed [13]. Natural products

provide an appreciable percentage of new active lead molecules, clinical candidates and drugs despite competition from different methods of drug discovery. The number of natural product derived drugs present in the total drug launches recently analyzed and it was concluded that natural products are still a significant source of new drugs, especially in different diseases like anti-cancer therapeutic areas [15, 16].

Anticancer activity of different extracts of *M. rosacea*, *A. marina* and *B. ceiba* was conducted with appropriate positive control (Adriamycin) which yielded results on HL60 cell lines, it was found that the ethyl acetate extract of *M. rosacea* exhibited more activity against HL60 cell lines but the inhibitory effect of this was observed to be a little bit weaker than that shown by Adriamycin and more activity compared to *A. marina* and *B. ceiba* plants extracts. From the results (Tables 1 and 2), it is clear that LD50 concentration of all fractions are greater than 80µg/ml i.e., the extract more preferably inhibiting the growth of cancer cells rather than killing the cells as the extract requires very high concentration to kill the cancerous

cells. While coming to growth inhibition, hydroalcoholic and ethyl acetate extracts of *M. rosacea* inhibited the HL60 cell lines better than the other extracts and the results are given in Table 1.

The ethyl acetate extract of *M. rosacea* showed total growth inhibition at 33.1µg/ml against HL 60 cell lines.

In case of GI 50 values, if it is $\leq 20\mu\text{g/ml}$ then it is considered to demonstrate the activity in case of extract. Here ethyl acetate extract of *M. rosacea* showed result i.e., 33.1µg/ml so it is considered as inactive on cancerous stem cells, but at the same time it was proved that a minimum of 40µg/ml concentration is sufficient to produce GI 50. From the above results it was clear that the plant *M. rosacea* was having cytotoxic activity against the HL 60 cell lines and supports the folkloric usage of the studied plant and confirmed that the studied plant possesses the constituents with cytotoxic properties that can be used for developing anticancer agents and if a pure compound isolated and checked for the activity may give the better results.

Table 1: Percentage (%) Control Growth of different extracts on HL60 cell lines

Human Leukemia Cell Line HL60																
% Control Growth																
Drug Concentrations (µg/ml)																
	Experiment 1				Experiment 2				Experiment 3				Average Values			
	10	20	40	80	10	20	40	80	10	20	40	80	10	20	40	80
SM_2	95.8	88.8	84.6	48.0	97.8	97.6	90.0	44.8	94.9	92.3	88.3	49.4	96.2	92.9	87.6	47.4
SA_1	100.0	100.0	100.0	94.5	100.0	100.0	100.0	85.3	100.0	100.0	95.1	90.4	100.0	100.0	98.4	90.1
SB_2	95.1	94.9	92.4	85.4	98.3	93.5	91.6	90.8	99.8	90.6	89.4	86.0	97.8	93.0	91.1	87.4
SM_1	97.9	52.1	15.0	4.5	94.3	55.1	13.8	2.7	81.0	50.5	16.6	5.9	91.1	52.6	15.2	4.4
SB_1	92.3	84.1	82.9	77.1	94.2	92.6	90.0	72.2	92.3	88.3	86.6	81.2	93.0	88.3	86.5	76.9
ADR	-7.3	-31.3	-46.6	-49.0	-4.7	-34.5	-34.5	-41.7	1.6	-12.7	-17.4	-26.8	-3.5	-26.2	-32.8	-39.2

Table 2: LC50, TGI and GI50 Drug concentrations (µg/ml) of different extracts on HL60 cell lines

Name of the extracts	Drug concentrations (µg/ml) calculated from graph		
	LC50	TGI	GI50
SM_2	>80	>80	>80
SA_1	>80	>80	>80
SB_2	>80	>80	>80
SM_1	>80	73.3	33.1
SB_1	>80	>80	>80
ADR	71.1	30.7	<10

GI50: Growth inhibition of 50 % (GI50) calculated from $[(Ti-Tz)/(C-Tz)] \times 100 = 50$, drug concentration resulting in a 50% reduction in the net protein increase

TGI: Drug concentration resulting in total growth inhibition (TGI) will be calculated from $Ti = Tz$

LC50: Concentration of drug resulting in a 50% reduction in the measured protein at the end of the drug treatment as compared to that at the beginning) indicating a net loss of 50% cells following treatment is calculated from $[(Ti-Tz)/Tz] \times 100 = -50$.

ADR: Adriamycin (Doxorubicin), Positive control compound.

GI50 value of $\leq 10^{-6}$ (i.e. 1 µmole) or $\leq 10\mu\text{g/ml}$ is considered to demonstrate activity in case of pure compounds. For extracts, GI50 value $\leq 20\mu\text{g/ml}$ is considered to demonstrate activity.

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