Influence of *Amoora rohituka* on MCF-7 Human Mammary Adenocarcinoma Cells *In Vitro*

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The ethanol extract of Amoora rohituka stem bark showed Cytotoxicity against MCF-7 cell lines derived from human mammary adenocarcinoma with a 50% inhibitory concentration (IC_{50}) of 30 μ/ml, but with no activity against HEp-2 from a tummour of the larynx.

IN Indian system of medicine, many plants are claimed to possess antitumour activity[1]. However, a very few of them have been rationally investigated to exploit their antitumour potential. **Amoora rohituka**(Syn.**Alphanamixis polystachya** N.O. Meliaceae) is a medicinal plant native of most of the hotter parts of India. It was employed locally as astringent and used in the diseases of spleen, liver and abdomen and also against tumours and rheumatism[2,3]. The powdered bark is used by Ayurvedic physicians in the treatment of enlarged spleen, in liver diseases, abdominal complaints and tumours[4]. However, it was found later to be unsatisfactory in enlarged spleen and enlargement of liver of infants[5]. Attempts were therefore made to investigate this plants antitumour potential.

The stem bark of **Amoora rohituka** used in this study was collected from the Ayurvedic Garden, Institute of Medical Sciences, Banaras Hindu University, Varanasi after identification with the aid of herbarium sheets of authentic species. The bark was washed, air- dried and powdered. The extraction was carried out using 20 gm of the dry powder with 200ml of ethanol and kept seven days at room temperature (35°c). the extract was evaporated under vacuum and dried to a constant weight at 37° C and then diluted with phosphate buffered saline (pH 7.4) to a concentration of 5 mg/ml and used for **in vitro** study.

Cell Cultures: MCF-7 derived from human mammary adenocarcinoma[6] in its 228th passage, HEp-2 from a tumour of the larynx[7] in its 189th passage were supplied by the National Facility for Animal Tissue and Cell Culture, Pune and the National Institute of Communicable Diseases, New Delhi respectively. Cells were grown in Eagle's minimum essential medium (MEM) supplemented with 10% fetal calf serum (FCS), 50 μ/ml streptomycin and 50 U/ml penicillin. The cells were incubated at 37°C in a humidified atmosphere containing 5% CO_2 and 95% air.

Cytotoxicity Assay: For growth inhibition studies, $1x10^4$ cells in expenential growth phase in 0.2 ml MEM, supplemented with 10% FCS were seeded into each well of 96 well plastic culture plates (Nunc. Denmark). The cells were incubated at 37°C in a humidified atmosphere containing 5% CO_2 and 95% air. After 48 hours cells were treated with the drug at various concentrations for 24 hours at 37°C in the same humidified atmosphere as mentioned above. After the medium was removed, cell monolayers were treated with trypsin and viable cell number was determined by a conventional haemocytometer using the trypan blue exclusion method. The IC_{50} values are the average of three assays.

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The Cytotoxic effects of the extract were examined in two humn cell lines, MCF-7 and HEp-2 derived from tumour tissues. **Table 1** summarizes the 50% inhibitory concentrations for cell growth (IC_{50}). The result indicated that a concentration of 30μ/ml was required for 50% cytotoxicity to MCF-7 cell line. There was a different level of susceptibility toward **Amoora rohituka** stem bark extract between the MCF-7 and HEp-2 cell lines, the former being sensitive and the latter relatively resistant.

Measurement of Cytotoxicity **in vitro** is a purely cellular event as presently carried out. For accepting **in vitro** testing as an alternative to animal testing, it must be demonstrated that potential cytotoxins reach the cells **in vitro** in the same form as they would **in vivo**. However, we have tried **in vitro** experiments as a convenient preliminary test for predicting the efficacy of the **Amoora rohituka** stem bark extract as an anticancer principle.

Table 1: In vitro cytotoxicity of the Amoora rohituka stem bark extract on MCF - 7 human mammary adenocarcinoma cells.

Conc. of extract μ/ml)	Percentage of live cells MCF-7
40	17.69 ± 1.04
*30	50.17 ± 3.59
20	71.89 ± 2.91
10	80.46 ± 2.39

Values are average of mean ± S.D. from 3 assays.

*IC_{50} : Concentration necessary to inhibit 50% of the cell growth.

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