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Research Article

IN VITRO EVALUATION OF CYTOTOXIC ACTIVITY OF FRUIT METHANOL EXTRACT OF *CERIOPS TAGAL* MANGROVE

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ABSTRACT

Anticancer activity of fruit methanol extract of *C. tagal* mangrove species against MDA-MB-231 (breast cancer) and HCT-116 (colon cancer) cell lines were studied. During the present investigation, crude fruit methanol extract of *C. tagal* was prepared using Soxhlet apparatus. *In vitro* anticancer activity of mangrove plant extract at various concentrations (5-320µg/mL) was studied against chosen cell line using MTT assay (3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide). The IC_{50} value for MDA-MB-231 and HCT-116 cell lines was 50.57µg/mL and 38.51µg/mL respectively. The present investigation indicates that fruit methanol extract of *C. tagal* is effective against tumor cells and possess anticancer activity.

Keywords: *Ceriops tagal*, cytotoxicity, MDA-MB-231, HCT116, scavenging activity, MTT assay.

INTRODUCTION

Cancer, which is defined as the uncontrolled growth of abnormal cells, is a major health problem. Cancer has now become the second major cause of human death. Therefore, there is a need to develop more effective and safe therapeutics, such as herbal remedies.

Mangroves are specialized group of salt tolerant plants that grow within the coast regions of tropic and sub tropic along the coastlines. They have distinctive characteristic like the ability to sustain in high salinity, extreme temperatures, anaerobic and unstable substrates thus forming unique environments and floral-faunal assemblages. Thus these plants produce special types of bioactive compounds than terrestrial plants.

Mangroves have been used in folk medicine for treatment of several diseases such as asthma, hepatitis, rheumatism, diabetes, diarrhea, small pox, leprosy etc.[1]. The mangroves are also known to have antimicrobial[2] antioxidant[3] properties. *Ceriopstagal* is a mangrove plant belonging to the Rhizophoraceae family has various medicinal uses. The decoction of the bark of *C. tagal* was used to treat hemorrhages and malignant ulcers in India[4], leaves are used to treat skin diseases, antifertility, diabetes, snake bite, asthma[5].The bark of this plant has been used for the treatment of infected wounds in Thailand and for obstetric and hemorrhagic conditions in the Philippines.

Several *in vitro* methods have been developed to measure the potency of natural anticancer compounds either as pure compounds or as crude plant extracts. *In vitro* methods like, Tryphan blue dye exclusion assay, LDH (Lactic dehydrogenase) assay, MTT assay, XTT assay and Sulforhodamine B assay are most commonly used for estimating anticancer properties of natural products from medicinal plants. Among these *in vitro* methods MTT is popular for estimating anticancer activity. In the present investigation cytotoxicity of *C. tagal* fruit methanol extract is studied. *C. tagal* species was also screened for antiviral[6], antioxidant[7] and cytotoxicity against HeLa human cervical carcinoma cancer cell line[8]. The anticancer principle in the roots of *C. tagal* was reported due to the presence of dolaborane diterpenes[9]. In the present study we have studied cytotoxicity of *C. tagal* fruit extract using MDA-MB-231 and HCT116 cell line by MTT assay.

MATERIALS AND METHODS

Plant material

C. tagal fruits were collected in the month of January from Gorai creek, Mumbai coast Maharashtra, India and identified by an expert taxonomist and deposited in the Dept. of Life Sciences, University of Mumbai.

Fruits were washed thoroughly under running tap water to free them from dust and other contaminants, oven dried at 40ºC to remove the moisture content, grinded, resultant powder was individually sieved through a muslin cloth and used for the study.

Extract Preparation

20 g *C. tagal* fruit powder was extracted by Soxhlet with 80% aqueous methanol. The extract solvent was completely evaporated by rotary evaporator to obtain sticky gummy residue. In 1g residue 10mL aqueous methanol (10%, v/v) was added and analyzed for cytotoxicity using MDA-MB-231(breast cancer) and HCT116 (colon cancer) cell line by MTT assay.

Cytotoxicity Assay by MTT Method
Procedure

The cells were collected when they reached about 70-80% confluency. The viability was checked and then centrifuged. Seeded 50,000 cells / well of MDA-MB-231 and HCT-116 in a

96 well plate separately and incubated for 24 h at 37ºC, 5% CO_2 incubator. Plant extract was added from (0 to 320µg/mL) (2 fold variation) concentration in RPMI (Roswell Park Memorial Institute medium) without FBS (Fetal Bovine Serum) and incubated for 24 h. The amount of MTT converted to formazan is a sign of the number of viable cells. After incubation with plant extract, 100L/well (50 µg /well) MTT (5 mg/10mL of MTT in 1X PBS) was added to the respective wells and incubated for 3 to 4 hrs. Then the MTT reagent was discarded by pipetting without disturbing cells and added 100 µL DMSO to rapidly solubilize the formazan and absorbance was measured at 590 nm using a micro plate reader. Cancer cells, which were cultured with medium in the absence of extract, were used as a control. All tests and analyses were carried out intriplicate. The 50% inhibitory concentration (IC_{50}) was defined as the concentration that reduced the absorbance of the untreated wells by 50% of the control in the MTT assay.

Calculating Inhibition:
The percentage growth inhibition was calculated as follows.
% Inhibition = (OD of Control - OD of sample/OD of Control) x 100

RESULTS

The effects of *C. tagal* fruit methanol extract on growth of cancer cells were examined by MTT assay. The viability rates of tumor cell in different concentrations are shown in table 1 and 2 for the MDA-MB-231 and HCT116 cell line respectively. It can be seen that the viability of treated cells decreased with the increasing concentrations. The IC_{50} for MDA-MB-231 and HCT-116 cell lines were 50.57µg/mL and 38.51µg/mL respectively.

DISCUSSION

In the present investigation we have evaluated the cytotoxicity from the fruit methanol extract of *C. tagal* plant using MTT assay and the results are shown in table (1-2). The cytotoxic activity was recorded on both MDA-MB-231 and HCT-116 cell lines by *C. tagal* fruit methanol extract. The activity increased as concentration increased. The best activity was recorded in HCT-116 (38.51µg/mL) than MDA-MB-231 (50.57µg/mL) indicating fruits are more potential against colon cancer than breast cancer.

A few mangroves species have shown anticancer properties. The *Acanthus ilicifolius* leaf and root indicated cytotoxic activity against MCF-7 (breast cancer) and PA-1(human ovarian teratocarcinoma) cell lines by MTT assay[10]. *In vitro* anticancer activity in *Avicennia marina* leaf extract on various cancer cell lines (HL-60, HepG2, NCI-H23 & HEK-293T) was also reported by MTT assay[11]. Beside this *in vitro* anticancer properties in *L. racemosa* and *Bruguiera sexangula* was also suggested [12-13]. All these reports are in agreement with the present findings. This suggests *in vitro* anticancer properties in mangrove species. The anticancer activity in the present study can be related to the presence of dolabranedinorditerpene and tagalsine principle in *C.tagal*[14]. Therefore *C. tagal* fruits can be used as potential source of anticancer activity.

Table. 1: Cytotoxicity of *C. tagal* fruit methanol extract against MDA-MB-231 cell line

MDA-MB-231				IC_{50} µg/mL
Plants name	**Conc. µg/mL**	**OD at 590 nm**	**% Inhibition**	
***C. tagal* fruit**	Control	0.6579	0.00	
	5	0.6425	2.34	50.57
	10	0.6249	5.02	
	20	0.5519	16.11	
	40	0.4549	30.86	
	80	0.3651	44.51	
	160	0.2937	55.36	
	320	0.2312	64.86	

Table. 2: Cytotoxicity activity of *C. tagal* fruit methanol extract against HCT-116 cell line

HCT-116				
Plants name	**Conc. µg/mL**	**OD at 590 nm**	**% Inhibition**	IC_{50} µg/mL
***C. tagal* fruit**	Control	0.8715	0.00	
	5	0.8207	5.83	38.51
	10	0.7266	16.63	
	20	0.6166	29.25	
	40	0.5135	41.08	
	80	0.3968	54.47	
	160	0.2708	68.93	
	320	0.2104	75.86	

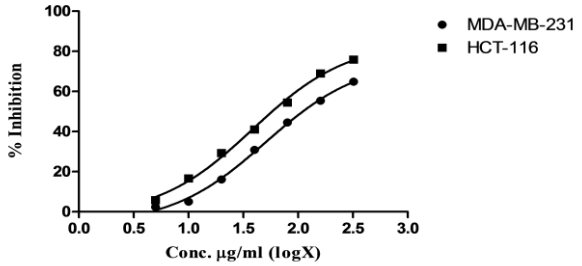

Figure 1: Growth Inhibition of *C. tagal* fruit methanol extract against MDA-MB-231 and HCT116 cell line

CONCLUSION

The crude fruit methanol extract of *C. tagal* showed good cytotoxicity against colon cancer. The extract also showed selective cell growth inhibition against selective cell lines. So, it could conclude that the fruit methanol extract of *C. tagal* can be very promising cytotoxic molecules that may lead novel formulation to treat various cancer malignancies in future especially in pharmaceutical and natural product studies but further studies are necessary for isolation and identification of biologically active substances.

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