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In-Vitro Cytotoxic Activity of Methanolic Extract of *Embelia tsjeriam Cottam* against Human Breast and Colon Cancer Cell Lines.

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ABSTRACT

The search for new anti-cancer drugs is one of the most prominent research areas of natural products. To investigate the cytotoxic potential of different extracts from *Embelia tsjeriam Cottam*. used in traditional medicine belonging to family Myrsinaceae against tumor cell lines in culture. Soxhlet extraction of roots of plant using methanol was carried out. The cytotoxicity of the extract was tested against human colon cancer (COLO-205) and breast cancer (MCF7) cell lines, using the Sulfarhodamine B assay. Tested methanolic extract demonstrated a substantial antiproliferative effect, inhibiting at least 50% of tumor cell proliferation at dose of 6.25- 400 μg/ml. The results of cytotoxicity studies indicate that *Embelia tsjeriam Cottam*. has remarkable cytotoxic activity and need to be further explored as a promising anticancer plant for clinical application in the future. Further studies of the active extracts are necessary for chemical characterization of the active compounds and more extensive biological evaluations.

Keywords: Anticancer activity, Growth inhibition, SRB assay, *Embelia tsjeriam cottam*.

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INTRODUCTION

Cancer is one of the most serious diseases that damage human health in the modern world and the second largest deadly disease just below heart disease [1]. According to the American Cancer Society deaths arising from cancer constitute 2–3% of the annual deaths recorded worldwide [2]. Currently available anticancer drugs like alkylating agents, anti-metabolites, apoptosis inducers, cell cycle inhibitors and hormonal regulators are harmful to the normal cells in great extent. So, there is an increased interest in new drug molecules of plant origin as they are less likely to cause serious side effects [3]. Over the past decade, herbal medicines have become a topic of global importance, making an impact on both world health and international trade. Medicinal plants continue to play a central role in the healthcare system of large proportions of the world's population [4].

Plants derived components have played an important role in the development of several clinically useful anticancer agents. These include vinblastine, vincristine, the camptothecin derivatives, topotecan and irinotecan, etoposide, derived from epipodophyllotoxin and paclitaxel (taxol) [5].

Embelia tsjeriam cottam belonging to the family Myrsinaceae, known as Baya-birang in Hindi is a climber found in the Western Ghats of Lonavala and also seen in the southern states of Maharashtra, Karnataka, Kerala, Tamil Nadu and Andhra Pradesh upto an altitude of 1600 m [6]. Dry fruit powder is used to oral contraceptive. Fresh fruits are eaten as raw for rheumatoid inflammation. The chemical constituents of this plant are embelin (2.5-3.1%), fatty ingredients: christembine- alkaloids, resinoids, tannins and volatile oils. Dried fruits are usually used as anthelmentic, astringent, carminative, taenifuge and stimulant [7]. It is given for the treatment of diarrhoea and cough. The dried bark of root is used for toothache [3-16]. The present research work was planned to evaluate the methanolic extract of the roots of *Embelia tsjeriam cottam* against the human colon cancer (COLO-205) and breast cancer (MCF7) cell lines using SRB assay.

MATERIAL AND METHODS

Chemicals

DMEM (Dulbecco's modified Eagle Medium), L-glutamine, penicillin and streptomycin were procured from INVITROGEN, Mumbai. FCS (Fetal calf serum) was purchased from SIGMA GIBCO.

Plant material

The plant was collected by in the months of July-Sept from Western Ghat region of Maharashtra state in India and it was taxonomically identified and confirmed by Dr. Bindu of Botany department of Mithibai College, SVKM campus Mumbai. The voucher botanic specimens were deposited at the Herbarium of said institution. The collected plant material was cleaned, shade dried at room temperature. The plant materials were coarsely powdered and stored in airtight containers.

Preparation of extracts

The surface sterilized, air-dried and powdered plant material was subjected to extraction with methanol using soxhlet apparatus. The crude extracts were obtained after the evaporation of the solvent under reduced pressure using rotary evaporator.

Assessment of cell viability

Cell culture

COLO-205 (Colon cancer), MCF7 (Breast cancer) cell lines were procured from NCCS, Pune. Cells were cultured at 37ºC in humidified CO_2 incubator with 5% CO_2 and 95 % air in their recommended medium [(DMEM for COLO-205 (Colon cancer), MCF7 (Breast cancer) cell lines] containing 10 % heat inactivated Fetal Calf Serum, 2 mM L- glutamine, 100 IU/ml penicillin and 100 µg/ml streptomycin. The cells were maintained by routine subculturing in 25 cm^2 plastic tissue culture flasks. 70-80% confluences of the cells were treated with Trypsin-EDTA for 5-7 minutes and sub cultured in new culture flask.

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Cytotoxicity Evaluation[17]

Cells were trysinized from flask showing 70-90% confluency. The suspension of single cell was checked for its viability and the cells per ml were counted. An appropriate volume of cells were added to centrifuge tube to get required cell density to add in 96-wellplate. A required volume of cell suspension of desired cell density was prepared for plating. 100 µl of cell suspension was added to each well. Frequent cell mixing was done to avoid variation in cell plating density in each well. 96-wellplate was then incubated for 24 hours at 37°C in 5% CO_2. A higher concentration of test extract and doxorubicin (standard) stocks were prepared in growth medium and sterilized through sterile syringe driven filter. For IC_{50} determination a two-fold serial dilutions from 200µg to 1.56µg/ml in 10 % v/v growth medium were made. 100 µl of testextract/ doxorubicin were added to wells (where n=3, n is number of wells foreach test extract /doxorubicin concentration). 96-wellplate was incubated for 48 hrs in CO_2 incubator at 37°C. 100 µl of tricloro aceticacid (10% w/v) were added to each well, 96-well plate was kept at 4°C for 1 hour for cell protein fixation. Plate was washed four times gently with deionized water. Plate was dried with air blower and 100 µl of Sulforhodamine was added to each well (0.057 %w/v concentration was optimized for Colo-205 while 0.4% w/v concentration was used for MCF-7 cell line as per protocol). 96-wellplate was washed immediately after 30 minutes with 1% acetic acid. 96-wellplate was dried with hotair blower. 200µl of Tris-base (10mM pH 10.5) was added and allowed to dissolve dye for 10-15 minutes by keeping it on shaker or 30 mins at room temperature. The 96-wellplate was read at 540 nm wavelength on microplate reader.

Percent growth inhibition was calculated by formula:

% Growth inhibition = (1- OD of Test/ OD of Negativecontrol) X 100

Where,
OD : Optical density.
Negative control: Cells + media
Positive control: Cells + different concentrations of Doxorubicin.
Test: Cells + Different concentrations of methanolic Extract of *Embelia tserium cottam*

RESULT AND DISCUSSION

Methanolic extract Embelia tsjeriam cottam of shows a significant reduction on MCF-7 and COLO-205 cell survival as shown in figure 1 and 2 respectively. The viability of MCF7 cells measured by the SRB assay was greatly reduced in a dose-dependent manner when cells treated with increasing doses (6.25- 400 µg/ml) of methanol extract and IC_{50} value was found to be 48.875 µg/ml. The viability of COLO-205 cells measured by the SRB assay was greatly reduced in a dose-dependent manner when cells treated with increasing doses (1.5-100 µg/ml) of methanol extract and IC_{50} value was found to be 48.65 µg/ml. Further studies with the extract may be of interest to identify new leads and compounds with cytotoxic properties.

Table 1: Percent cytotoxicity of methaolic extract of *Embelia tsjerium cottam* roots against MCF -7 cell line

Conc. (ppm)	% Growth inhibition
6.25	45.897
12.5	46.978
25	48.214
50	51.108
100	54.215
200	61.824
400	78.382

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Table 2: Percent cytotoxicity of methanolic extract of *Embelia tsjerium cottam* roots against COLO-205 cell line

Conc.µg/ml	% Growth inhibition
1.5625	30.9876
3.125	31.4804
6.25	33.2789
12.5	36.0649
25	41.208
50	51.0147
100	69.9839
IC50	48.6582278

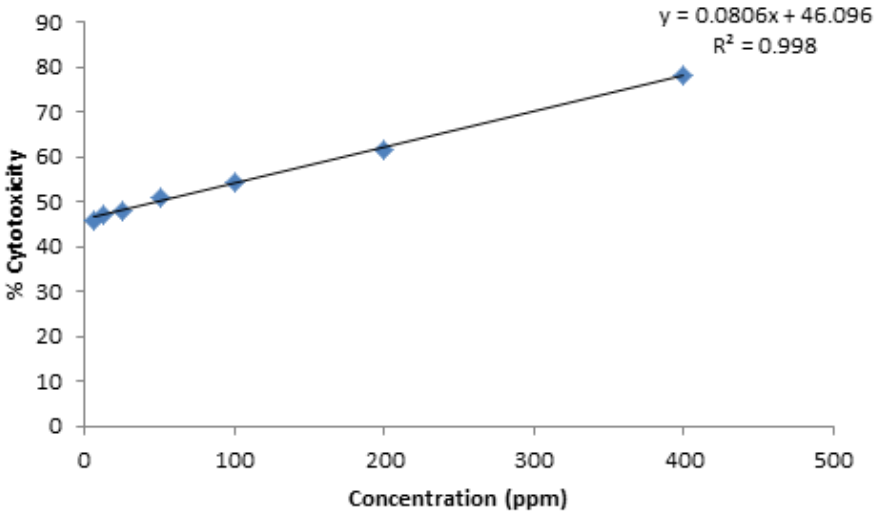

Figure 1: Plot of percent cytotoxicity methanolic extract of *Embelia tsjerium cottam* roots against MCF -7 cell line

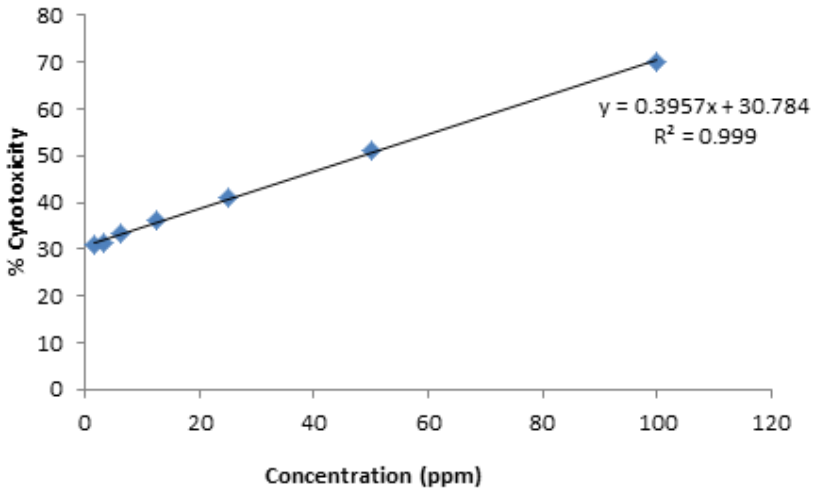

Figure 2: Plot of percent cytotoxicity methanolic extract of *Embelia tsjerium cottam* roots against COLO-205 cell line

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