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RESEARCH ARTICLE

PRELIMINARY STUDIES ON *IN VITRO* ANTI-TUMOR ACTIVITY OF TENDER SEED COAT EXTRACT OF *Borassus flabellifer* L. ON HELA CELL LINE

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ABSTRACT

The study was aimed to evaluate the anticancer activity of the seed coat of *Borassus flabellifer* on the HeLa cell line. The seed coat of *Borassus flabellifer* PBS extracts were tested for inhibitory effect on HeLa Cell Line. The cytotoxicity of *Borassus flabellifer* on HeLa cell was evaluated by the MTT assay. The PBS extract of *Borassus flabellifer* has shown significant cytotoxicity on HeLa Cell Line in concentration range between 32 µg/ml to 750 µg/ml by MTT assay. Our preliminary studies on HeLa cell line indicated that even the lower concentrations of plant extract tested showed significant antiproliferative activity.

INTRODUCTION

Cancer is a major public health burden in both developed and developing countries. Cancer is a leading cause of death worldwide: it accounted for 7.9 million deaths (around 13% of all deaths) in 2007. Deaths from cancer worldwide are projected to continue rising, with an estimated 12 million deaths in 2030. Cancer is one of the most serious threats to human health in the world and chemotherapy is still the standard treatment method. Most of the anticancer drugs currently used in chemotherapy are cytotoxic to normal cells and cause immunotoxicity which affects not only tumor development, but also aggravates patient's recovery. The discovery and identification of new antitumor drug with low side effects on immune system has become an essential goal. With this aim, many attentions have been paid to natural compounds in plants, marine and microorganisms.

Plants have many phytochemicals with various bioactivities, including anticancer activity. Several anticancerous compounds are derived from plant sources and some of them are in clinical use. For example, two alkaloids vinblastine and vincristine were isolated from *Catharanthus roseus* (Apocynaceae) (Jordon *et al.,* 1991). Another natural product, paclitaxel that has been isolated from the bark of Pacific Yew, *Taxus brevifolia* (Taxaceae) (Fuchs and Johnson, 1978), while *Taxus baccata* was being used in Indian Ayurvedic system for the treatment of cancer. Camptothecin was isolated from the Chinese ornamental tree *Camptotheca acuminate* (Nyssaceae), found to be highly cytotoxic in nature (Wall *et al.,* 1966). Podophyllotoxin was isolated as the active anti-tumor agent from the roots of *Podophyllum peltatum* and *Podophyllum emodi* (Berberidaceae) (Gordaliza *et al.,* 2004). Homo harringtonine, isolated from the Chinese tree *Cephalotaxus harringtonia* (Cephalotaxaceae), is another plant-derived agent in clinical use (Powell *et al.,* 1972).

Numerous types of bioactive compounds have been isolated from plant sources. Several of them are currently in clinical trials or preclinical trials or undergoing further investigation. A plant alkaloid rohitukine, which was isolated from *Dysoxylum binectariferum* (Meliaceae) reported to possess antitumor activity (Mohanakumara *et al.,* 2010). Another natural product olomucine, originally isolated from *Raphanus sativus* (Brassicaceae) is in Phase II clinical trials (Gordon and David, 2005). Combretastatins were isolated from the bark of the South African tree *Combretum caffrum* (Combretaceae) and were highly cytotoxic in nature (Daniele *et al.,* 2006). Betulinic acid, a pentacyclic triterpene was isolated from *Zizyphus* species and displayed selective cytotoxicity against human melanoma cell lines (Pisha *et al.,* 1995). Pervilleine A was isolated from the roots of *Erythroxylum pervillei* Baill. (Erythroxylaceae) was selectively cytotoxic against oral

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epidermoid cancer cell line (KB-V1) (Mi *et al.,* 2003). Silvestrol was first isolated from the fruits of *Aglaila sylvestre* (Meliaceae) exhibited cytotoxicity against lung and breast cancer cell lines (Mohammad Shoeb, 2006). Two novel alkaloids, schischkinnin and montamine have been isolated from the seeds of *Centaurea schischkinii* and *Centaurea montana* exhibited significant cytotoxicity against human colon cancer cell lines (Mohammad Shoeb, 2006). Reports are not available on antitumor activity of the seed coat of *Borassus flabellifer*. Therefore, the present study has been undertaken to investigate the antitumor activity against HeLa cell line.

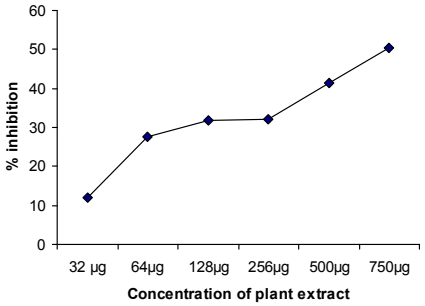

Fig 1. Dose-response curve of the *Borassus flabellifer* seed coat extract on the growth of HeLa cells cultured in vitro

MATERIAL AND METHODS

Preparation of plant extract

Borassus flabellifer tender seeds locally termed as '*Thati munjelu*' were obtained from local market in the summer season from Visakhapatnam, Andhra Pradesh. Tender seed coat of *Borassus flabellifer* is removed and air dried then ground into powder which (1mg/1ml) was dissolved in phosphate buffered saline then centrifuged to remove the debris. Finally supernatant was collected and tested for antitumor activity.

Cell line and culture medium

Carcinoma of cervix (HeLa) cells were obtained from TRIMS, Visakhapatnam, India. They were cultured in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 4.5 g/L glucose, 2 mM L-glutamine and 5% fetal bovine serum (FBS) at 37°C in 5% CO_2 incubator.

MTT assay for antitumor activity

The MTT assay developed by Mosmann (Mosmann, 1983) was modified and used to determine the inhibitory effects of seed coat extracts on cell growth in vitro. In brief, the trypsinized cells from T-25 flask were seeded in each well of 96-well flat-bottomed tissue culture plate at a density of $5x10^3$ cells/well in growth medium and cultured at 37°C in 5% CO_2 to adhere. After 48hr incubation, the supernatant was discarded and the cells were pretreated with growth medium and were subsequently mixed with different concentrations of seed coat extracts (32, 64, 128, 256, 500 and 750 µg/ml) in triplicates to achieve a final volume of 100 µl and then cultured for 48 hr. The compound was prepared as 1.0 mg/ml concentration stock solutions in PBS. Culture medium and solvent are used as controls. Each well then received 5 µl of fresh MTT (0.5mg/ml in PBS) followed by incubation for 2hr at 37°C. The supernatant growth medium was removed from the wells and replaced with 100 µl of DMSO to solubilize the colored formazan product. After 30 min incubation, the absorbance (OD) of the culture plate was read at a wavelength of 570 nm by Anthos 2020 microplate reader, UK.

RESULTS AND DISCUSSION

Determination of antitumor activity of *B. flabellifer* seed coat extract by MTT assay

Borassus flabellifer seed coat extracts were screened for their possible anticancer activity on growth of the HeLa cells in vitro in presence or absence of the plant extract. The phosphate buffered saline (PBS) extract of seed coat of *Borassus flabellifer* were administered at different cocentrations viz., 32, 64, 128, 256, 500 and 750 µg/ml and found that the growth of the HeLa cells was significantly inhibited.

Table 1. Optical density and percent inhibition of HeLa cell growth at various concentrations of *Borassus flabellifer* extract

Seed coats extract concentration (µg/ml)	Observed OD* at 570nm	% Viability	% Inhibition
Control	0.725	100	0
32	0.642	87.95	12.05
64	0.528	72.33	27.67
128	0.498	68.22	31.78
256	0.496	67.95	32.05
500	0.427	58.49	41.51
750	0.362	49.59	50.41

* Values are mean of triplicates

Moreover, the inhibitory activity of *Borassus flabellifer* extract was found to be dose-dependent as the percentage of inhibition of HeLa cell growth increases upon increasing the concentration of the extract (Fig 1.). As shown in the Table 1., 50% inhibitory effect was found with 750 µg/ml seed coat crude extract indicating the significance of antiproliferative activity. Preliminary phytochemical analysis and from spectral characteristics of aqueous extract of *Borassus flabellifer* (data not shown) indicate the presence of steroidal saponins and that may be accountable for these activities. However further investigations are needed to isolate and further characterize the antitumor compound from the seed coat of *Borassus flabellifer*.

Conclusion

Natural products discovered from medicinal plants have played an important role in the treatment of cancer. Several plants or plant products have been screened for antitumor activity. Several indigenous and ethnomedicinal plants provide immense scope to explore antitumor molecules. Our preliminary studies revealed that the *Borassus flabellifer* seed coat contain anti-proliferative compounds. We are in the process of isolating and characterizing the antitumor molecule and to examine its efficacy on different cell lines.

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