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In vitro cytotoxic activity of rhizome extracts of *Cyperus rotundus* (L.) against colon carcinoma and Ehrlich ascites carcinoma

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ABSTRACT

Cyperus rotundus (L.) is a perennial herb belonging to family Cyperaceae having tremendous medicinal properties and it has been used in various formulations of Indian system of medicine. This study was designed to investigate the *in vitro* anticancer potential of aqueous and ethanol extracts of *Cyperus rotundus* (L.).The cytotoxicity was assessed using SRB Assay and Trypan blue assay against human colon cancer cell line (HCT-116) and Ehrlich Ascites Carcinoma (EAC) cell lines. Results of SRB assay on HCT-116 cells indicated an IC_{50} value of 517.828 µg/ml for aqueous extract and that 72.06 µg/ml for ethanol extract. In trypan blue assay, IC_{50} values of 2158.63 µg/ml and 160.19 µg/ml were obtained for aqueous and ethanol extracts respectively on EAC cells. Positive control Doxorubicin exhibited IC_{50} of 11.221 µg/ml and 23.325 µg/ml against HCT-116 and EAC cells respectively. Findings from this study prospect the possible use of *C. rotundus* as a potential source of anticancer agent.

INTRODUCTION

Cancer is the second most leading cause of death while heart disease being the first in the world (Jemal *et al*., 2007). Cancer accounted for 8.2 million deaths in 2012. Nearly 30% of deaths from cancer are due to high body mass index, low intake of fruits and vegetables, lack of physical activity, use of tobacco/alcohol and environmental effects. In developing countries, mortality rates are higher due to lack of healthcare facility and greater exposure to carcinogens 2012 (Steliarova *et al*., 2012). Despite good progress in the diagnosis and treatment for cancer, it is a major threat accounting for 23% of the deaths in the USA and 7% deaths in our country (American Cancer Society, 2015). Existing therapeutic modalities pose a magnitude of side effects, thereby inculcating the need for search of alternative therapies. Herbal medicine plays a vital role in traditional method of healing worldwide against cancer. Plant products, due to their antioxidant property can modulate the physiology as well as metabolism effectively to significantly reduce the amount of free radicals and reactive oxygen species generated in tumor microenvironment. Due to the medicinal property of phytochemicals, they are used during or after the cancer therapy treatment to neutralize damage and harmful consequences due to cancer therapies. Use of phytochemicals has long-term health benefits; however, they are used in parallel to the cancer therapy because cancer therapy can cause significant damage to the tumor cells within a short period of time (Eva *et al*., 2006). *Cyperus rotundus*, commonly known as Musta, is a medicinal plant belongs to family Cyperaceae. In Indian system of medicine, its rhizomes are used in the treatment of several clinical conditions like candidiasis, diabetes, diarrhea, malaria, dysmenorrhea and menstrual irregularities (Sivapalan *et al*., 2013). Its oil extracts are widely used in ancient medicine for various kinds of health problems like stomach problem, constipation, fever, tooth problems and digestive disorders antispasmodic and menstrual irregularities (Puratchikody *et al*., 2006). Whole plant and closer view of the rhizome is presented in Fig. 1. Proposed study was designed to investigate the *in vitro* cytotoxic potential of aqueous and ethanol extracts of *Cyperus rotundus* (L.) against colon cancer and EAC cells.

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MATERIALS AND METHODS

Collection and identification of the herb

Dried rhizomes of *C.rotundus* were collected from a local Ayurvedic pharmacy in Mangalore, Karnataka, India. The plant material was authenticated by Dr. Sunil Kumar, Senior Research officer, Department of Pharmacognosy, SDM Centre for Research in Ayurveda and Allied Sciences, Udupi and voucher specimen (No.11110101) was deposited in the plant repository of SDM Research Center. The shade dried rhizomes of the *C. rotundus* were coarsely powdered and preserved at -20^{o} C for further studies.

Fig. 1: *Cyperus rotundus*. A: Whole plant; B: Closer view of rhizome.

Preparation of extracts

Ethanol and aqueous extracts of *C. rotundus* were prepared as per the standard procedures (Raman, 2006).

Cell lines and culture conditions

Human colon cancer cell line (HCT-116) was procured from National Centre for Cell Sciences, Pune. Ehrlich's Ascites carcinoma (EAC) were obtained from Amala Cancer Research Center, Amala Nagar, Thrissur, Kerala, India. The cells were maintained as ascites tumor in Swiss albino mice by *i.p.* inoculation.

Dulbecco's modified eagles media (DMEM), RPMI-1640, fetal bovine serum (FBS) and SRB reagent were procured from Sigma Aldrich, USA. HCT-116 cells were cultured in DMEM supplemented with 10% FBS and 1% antibiotic solution. EAC cells were cultured in RPMI-1640 supplemented with 10% FBS. Cells were maintained at 37^{0}C and 5% CO_2 in a humidified atmosphere. They were used for the experiments after three consecutive passages.

Sulphorhodamine-B (SRB) assay

HCT-116 and EAC cells were seeded onto 96 well flat bottom microtiter plates at a seeding density of 5 x10^{3} cells in 0.1 ml of DMEM medium supplemented with 10% FBS and allowed to attach for 24 h. Test compounds were prepared just prior to the experiment and serially diluted with medium to get the working stock of 200, 100, 50 and 25 µg/ml concentration. After 24 hrs of incubation, cells were treated with 100 µl of test solutions at different concentrations and further incubated for 48 h. Cells in the control group received medium only containing 0.5, 0.25 % DMSO. Each treatment was performed in triplicates. After the treatment duration, drug containing media was removed and washed with 200 µl of PBS.50 µl of 30 % TCA was added to fix the cells and kept at 4^{0} C for 1 h, following which each well was gently washed with sterile water to remove TCA, medium and dead cells. Plate was allowed to air dry and 50µl of 0.05% SRB dye solution was added and incubated at room temperature in dark for 30 min.After 30 min, unbound SRB was removed by washing with 200 µl of 1% v/v acetic acid. The plate was air dried and 200 µl of trizma base buffer was added to each well to dissolve the cell bound dye.The plate was shaken for 20 min on a gyratory shaker and the optical density was recorded at 540 nm with reference wavelength of 630 nm in an ELISA plate reader. Percentage cell viability was calculated. Values were represented as Mean ± SEM. O.D values (proportional to cell death) were plotted against the tested drug concentrations.

Trypan blue exclusion assay

Stock cell suspension at a density of 1X10^{7} was made in PBS, from which 100 µl of suspension was taken in sterile test tubes. The cells were treated with 100 µl of test drugs of varying concentrations (not more than 0.1% DMSO) and 700 µl of PBS was added. Cells were incubated at 37^{0}C for 3 h. After the exposure 100 µl of trypan blue was added and mixed well. Total numbers of dead and viable cells in all the four corner squares of the chamber were counted using a haemocytometer and the percentage viability was calculated as follows:

% viable cells = (Number of unstained cells/Total number of cells) x 100

Statistical Analysis

All data were presented as Mean ± SEM. The significance of intergroup differences was evaluated by one-way ANOVA followed by Dunnett's t-test using Graph Pad Prism version 5.0. Statistical differences were considered significant at $p < 0.05$.

RESULTS AND DISCUSSION

Evaluation of anticancer activity of *C. rotundus* extract by SRB assay revealed that ethanol extract possessed potent anticancer activity with an IC_{50} value of 72.06 µg/ml on HCT-116 cells after 48 h of exposure (Fig. 2). The standard used was Doxorubicin which showed an IC_{50}value of 11.221 µg/ml.

Fig. 3 depicts the *in vitro* cytotoxic effect of aqueous and ethanol extracts of *C. rotundus* on EAC cell line by Trypan blue dye exclusion assay. IC_{50} value was found to be 2158.653 and 160.190 µg/ml for aqueous and ethanol extracts respectively. The standard drug Doxorubicin showed an IC_{50}value of 23.375 µg/ml.

The present investigation showed that ethanol extract of *C. rotundus* acted as a potential cytotoxic agent on both colon carcinoma and EAC. Several phytochemicals from natural sources have been effectively used for the treatment of cancer. Among the arena of phytochemicals which are under clinical trials to be used

as alternatives to chemopreventive agents, apigenin from parsley, curcumin from turmeric, crocetin from saffron, cyanidins and resveratrol from grapes, diindolylmethane (DIM) from Brassica vegetables, epigallocatechin gallate from green tea, fisetin from strawberries and apples, genistein from soybean, gingerol from gingers, kaempferol from tea and broccoli, lycopene from tomato, phenyl isothiocyanate and sulforaphane from cruciferous vegetables, rosmarinic acid from rosemary, triterpenoids from wax-like coatings of fruits and medicinal herbs, Vitamin D from mushroom and Vitamin E from plant oil are the major ones (Nirmala *et al*., 2011; Liu, 2004; Solanki, 2004). Phytochemical screening of both extracts indicated the presence of coumarins, carbohydrates, steroids, phenols and saponins. In ethanol extract, terpinoids and tannins were also present.

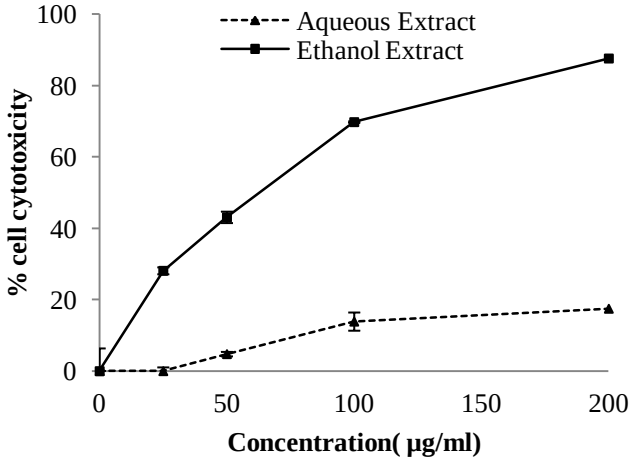

Fig. 2: *In vitro* cytotoxic effect of aqueous and ethanol extracts of *C. rotundus* on HCT 116 cells by SRB assay.

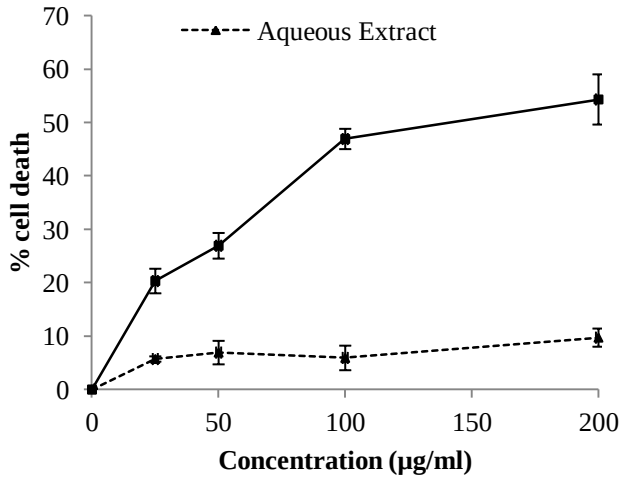

Fig. 3: *In vitro* cytotoxic effect of aqueous and ethanol extracts of *C. rotundus* on EAC cell line by Trypan blue dye exclusion assay

Cyperus rotundus, a perennial grass, has long been used as herbal medicine for several diseases and health problems due to its anti-microbial, anti-malarial and anti-spasmodic properties (Zhu *et al.,* 1997; Bhattarai, 1993; Uddin *et al*., 2006). Essential oils, flavonoids, terpenoids, mono and sesquiterpenes of *Cyperus* were recently found to have significant antioxidant, free-radical scavenging activity and showed inhibitory action against lipid peroxidation, protein oxidation and glycoxidation (Yazdanparast and Ardestani, 2007). Extracts from *C. rotundus* showing anti-mutagenic and radical scavenging activities against aflatoxin B1 and sodium azide were also reported (Kilani *et al*., 2005). A recent study proved cytotoxic and anti-apoptotic activity of *C.rotundus* tuber extract in murine leukemic cell lines L1210 (Kilani *et al.,* 2008). Findings for this investigation prospect *C. rotundus* ethanol extractives as a potential anticancer agent after pre-clinical and clinical investigations.

CONCLUSION

This study provides the cytotoxic activity of rhizome extract of *C. rotundus*. The anticancer activity of C. *rotundus* ethanol extract was higher than that of the aqueous extract, thereby indicating its possible usage as a potential source of cancer therapeutic. Active principles from *C. rotundus* ethanol extract can be used as ingredients of anticancer formulations.

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