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POTENT SELECTIVE CYTOTOXIC ACTIVITY OF KAEMPFERIA GALANGA L. RHIZOME AGAINST CANCER CELL CULTURES

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ABSTRACT

Crude ethanol extract of the rhizome *Kaempferia galanga* L. and the successive extracts prepared with increasing polarity viz., petroleum ether, ethyl acetate and ethanol. The extracts and fractions were screened for cytotoxic activities by standard MTT and SRB assays against four cancerous viz., DU145, PA1, SW620, B16F10 and a normal Vero cell cultures. The successive ethyl acetate extract showed selective toxicity towards cancer cells and showed less toxicity towards normal cells. The successive petroleum ether fraction showed potent activity against human colorectal adenocarcinoma cells with CTC_{50} 0.55 µg/ml and was comparatively non toxic towards normal Vero cell cultures.

KEY WORDS

Cytotoxicity, MTT assay, SRB assay, *Kaemperia galanga*

INTRODUCTION

Natural products are known to provide lead compounds in the past and play a significant role in future in the treatment of cancer. *Kaemperia galanga* L. (Zingiberaceae) is an acaulescent perennial that grows in southern China, Indochina, Malaysia, India and Bangladesh[1]. The rhizome of this plant has been used traditionally for the treatment of many ailment and few biological activities have proven its importance. The rhizome is rich in essential oils and is being used traditionally for the treatment of indigestion, cold, pectoral and abdominal pains, headache, expectorant, diuretic and carminative[2], skin disorders, rheumatism and diabetes mellitus[3,4]. The antihypertensive[5] and larvicidal activity[6] of the rhizome have been reported. However a systematic study to reveal its cytotoxicity against normal and cancer cells have not undertaken yet. Hence, in the present study we report the cytotoxic activity of the crude alcoholic extract and successive extracts of rhizome of this plant in both normal and cancer cell cultures.

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MATERIALS AND METHODS

Chemicals

3-(4,5dimethyl thiazole-2 yl)-2,5-Diphenyl tetrazolium bromide (MTT), Sulphorhodamine B (SRB), Trypan blue and F-12 Coon's medium were obtained from Sigma Chemical Co., Mo, U.S.A. DMEM, phosphate buffered saline (PBS) and antibiotics used were obtained from Hi-media Ltd., Mumbai, India. Trichloro acetic acid (TCA) and tris buffer were obtained from SD fine chemicals Pvt. Ltd., Boisar, India. DMSO, glacial acetic acid and propanol were obtained from E.Merck Ltd., Mumbai, India.

Cell lines and culture medium

Cell lines representing SW 620 (human colorectal adenocarcinama cells), PA-1 (human ovarian teratocarcinoma cells), DU-145 (human epithelial prostrate cancer cells), B16-F10 (mouse skin melanoma cells) and Vero (normal, African green monkey kidney) were selected for the study. The cultures were maintained in Dulbecco's Minimum Essential Medium (DMEM) containing 10% inactivated new born calf serum (NBCS) and were grown in 25 cm^2 tissue culture flasks (Tarsons Products (P) Ltd., Kolkotta) till confluent and used for cytotoxicity assays.

Plant Materials

The rhizome of *Kaemperia galanga* was purchased from Abirami Botanicals, Tuticorin, Tamilnadu, India and authenticated by Professor of Botany, Mahatma Gandhi Memorial College, Udupi, India

Preparation of crude and successive extracts

The powdered rhizomes (250 g) were also subjected to extraction with ethanol in a soxhlet extractor for 18-20 h. The extract was concentrated, to yield a brownish black coloured semisolid residue (KG) with the yield of about 4% w/w. The shade dried rhizomes were crushed into small pieces and coarsely powdered and extracted (250 g) successively with petroleum ether (60-80^oC), ethyl acetate and ethanol in a soxhlet extractor for 18-20 h. The extracts were concentrated to dryness in a rotary vacuum evaporator under reduced pressure and controlled temperature (40-50^oC). The petroleum ether extract (SKG-1) yielded a golden yellowish oily liquid, the ethyl acetate extract (SKG-2) yielded a dark brownish semi solid residue and alcoholic extracts (SKG-3) yielded a black semi solid residue. The yields were about 3.5, 2.5, and 3 % w/w with respect to dried powdered material of rhizome.

Accurately weighed extracts were dissolved separately in distilled dimethyl sulfoxide (DMSO) and the volume was made up to 10 ml with DMEM with 2% NBCS to obtain a stock solution of 1 mg/ml concentration and stored at -20 ^oC until further use.

Cytotoxicity assay

The cytotoxic assays were carried out using 0.1 ml of cell suspension, containing 10,000 cells seeded in each well of a 96 well microtitre plate. Fresh medium containing different concentrations of the Extracts was added to the wells 24 h after seeding. Control cells were incubated without the test solutions and with DMSO solvent. The very little percentage of DMSO present in the wells (maximal 0.2%) was proved not to affect the experiment. The microtitre plates were incubated at 37^oC in a humidified incubator with 5% CO_2 for a period of three days. Eight wells were used for each concentration of the extracts. Morphological changes were recorded using an inverted microscope. The cells were observed at different time intervals after incubation in the

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presence or absence of the test solutions. Cellular viability was determined by using the standard MTT[7] and SRB[8] assays from the treated culture of 4 wells of each concentration. The percentage inhibition was plotted against concentration and CTC_{50} (concentration required to reduce viability by 50%) value for each cell line was calculated.

RESULTS AND DISCUSSION

Among the four extracts tested for cytotoxicity against cancer and normal cell cultures, successive ethyl acetate extract of *Kaemperia galanga* (SKG 2) showed selective toxicity against all the four cancer cells tested (Table 1). It showed less toxicity towards normal kidney cell culture with average CTC_{50} value 41.11 µg/ml. The average CTC_{50} values obtained after performing MTT and SRB assays were 2.93, 5.75, 8.29 and 2.22 µg/ml against cancer cells DU 145, PA 1, SW 620 and $B_{16}F_{10}$ cells, respectively. The successive petroleum ether extract, SKG 1 also showed selective toxicity against human colorectal adenocarcinoma cells with CTC_{50} value 0.55 µg/ml when compared to normal Vero cells 16.75 µg/ml. All the other extracts showed moderate cytotoxicity and were not selective against cancer cells.

The results of cytotoxicity studies and the CTC_{50} values thus obtained, indicate potent selective toxicity property of ethyl acetate fraction of *Kaemperia galang* rhizomes against cancer cells. The other tested extracts are cytotoxic only when the cultures are exposed to very high concentrations. The preliminary phytochemical studies of petroleum ether and ethyl acetate successive extracts showed the presence of terpenes. Several terpenes from the rhizome of this plant viz., pinene, camphene, carvone, benzene, eucalyptol, borneol, methyl cinnamate, pentadecane and ethyl-*p*-methoxycinnamate have been isolated. Among them the cytotoxic and anticancer activity of ethyl-*p*-methoxycinnamate is known[9]. Hence ethyl acetate fraction rich in terpenoids of *Kaemperia galanga* rhizomes merits further investigations to explore its anticancer activity.

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Table 1.

Cell cultures	Cytotoxicity ($^{\#}CTC_{50}$) ± S.E.M. (µg/ml)*											
	SKG-1			SKG-2			SKG-3			KG		
	MTT	SRB	Avg.	MTT	SRB	Avg.	MTT	SRB	Avg.	MTT	SRB	Avg.
Vero	15.63 ± 0.37	17.86 ± 0.53	16.75	39.84 ± 1.36	42.38 ± 1.94	41.11	59.35 ± 2.37	60.29 ± 2.93	59.82	10.52 ± 0.53	11.05 ± 0.21	10.79
DU 145	11.56 ± 0.47	13.53 ± 1.37	12.55	2.50 ± 0.22	3.36 ± 0.31	2.93	52.26 ± 2.24	51.21 ± 2.27	51.74	10.51 ± 0.34	11.22 ± 0.27	10.87
PA1	13.11 ± 0.33	14.83 ± 0.67	13.97	5.26 ± 0.56	6.24 ± 0.28	5.75	36.16 ± 2.53	39.93 ± 2.11	38.05	10.53 ± 0.22	11.36 ± 0.39	10.95
SW620	0.51 ± 0.01	0.59 ± 0.01	0.55	8.53 ± 0.72	8.04 ± 0.51	8.29	22.12 ± 1.03	23.87 ± 1.21	23.00	6.13 ± 0.52	6.39 ± 0.92	6.26
B16F10	14.37 ± 0.33	13.95 ± 0.85	14.16	2.08 ± 0.03	2.35 ± 0.03	2.22	44.19 ± 2.43	46.87 ± 2.13	45.53	12.63 ± 1.24	13.34 ± 0.95	12.99

Cytotoxic activity of Kaemperia galanga rhizomes on normal and cancer cell lines by MTT and SRB assays

* Average of three independent determinations, 4 replicates, values are mean ± SEM, #CTC_{50} = concentration of the sample required to kill 50% of the cells.

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REFERENCES

1. Kanjanpothi D, Panthong A, Lertprasertsuke N, Taesotikul T, Rujjanawate C, Kaewpinit D, Sudthayakorn R, Choochote W, Chaithong U, Jittpakdi A, Pitasawat B. Toxicity of crude rhizome extract *Kaempferia galanga* L. (Prph Hom). J. Ethno Pharm.: 90: 359-365, (2004).
2. Achuthan CR, Jose Padikkala. Hypolipidemic effect of *Alpinia galanga* (Rasna) and *Kaempferia galanga* (Kachoori). Ind J Clin Biochem 12: 55–58, (1997)
3. Mangaly JK, Sabu M. Ethanobotany of Zingiberaceae. Zingiberaceae workshop. Prince of Ongkla University, Hat Yai, Thailand.: 15–18, (1991)
4. Vagbhata. Astanga Hridayam Chikitsa Stanum (Saranga Sundara Commentary of Arun Datta and Hemadri) 3rd Chapter. Chaukhambha Orientalia,Varanasi, Delhi 1971: 167–681.
5. Othman R, Ibrahim H, Mohd MA, Awang K, Gilani AV, Musthafa MR. Vaso relaxant effect of *Kaempferia galanga* on smooth muscles of rat aorta. Planta Med 68: 655–657, (2002)
6. Choochote W, Kanjanapothi D, Panthanga A, Taesotikul T, Jitpakdi A, Chaithong U, Pitasawat B. Larvicidal and repellent effects of *Kaempferia galanga*. Southeast Asian J Trap Med Public Health 30: 470–476, (1999)
7. Francis D, Rita L. Rapid colorimetric assay for cell growth and survival: modifications to the tetrazolium dye procedure giving improved sensitivity and reliability. J. Immunol. Methods 89: 271-277, (1986)
8. Philip S, Rista S, Dominic S, Anne M, James M, David V, Jonathan TW, Heidi B, Susan K, Michale RB. New colorimetric cytotoxic assay for anti cancer drug screening. J. Natl. Cancer Inst. 82: 1107-1112, (1990)
9. Zheng GQ, Kenny PM, Lam LKT. Potential anticarcinogenic natural products isolated from lemon grass oil and galanga root oil. J. Agric. & Food Chem. 41: 153-156, (1993)