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Preliminary Anticancer Screening and Standardization of some Indigenous Medicinal Plants using Cell-biology and Molecular Biotechnology Based Models

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ABSTRACT

Alcoholic extracts of four Indian medicinal plants Kaempferia galanga (*Zingiberacae*) Linn. *Clerodendrum viscosum* (Verbenaceae) Linn., Jatropha curcus (Euphorbiaceae) Linn. and *Lens culinaris* (Fabaceae) Linn. were subjected to preliminary screening for their antitumor activity. Acute toxicity studies in mice revealed that all the ethanolic extracts were safe up to a dose level of 500, 1000, 2000 mg kg^{-1} body weight. Preliminary short term anticancer screening, by brine shrimp lethality test, potato disc inhibition and DLA cell line assay, proved that *K. galanga*, exhibited significant antitumor activity and it was therefore, selected as a candidate plant for more detailed phytochemical and mechanistic studies. Brine shrimp lethality assay revealed that *K. galanga* extract inhibited tumor development at a lower concentration LC_{50} = 684.2 μg mL^{-1} as compared to 901, 866 and 5436 μg mL^{-1} for the extracts of *C. viscosum, J. curcus* and *L. culnaris*, respectively. Alcoholic extract of *K. galanga* significantly ($p<0.001$) inhibited *Agrobacterium* induced tumors in potato discs with average tumor count of 15, 11, 8.0, 6.0 and 4.8 at concentrations of 10, 20, 30, 40 and 50 μg mL^{-1}, respectively. *K. galanga* extract regress tumors equipotently to vincristine in Dalton Lymphoma Ascitic (DLA) cell tumor bearing mice. There was a statistically significant ($p<0.001$) higher mean increase in Percentage Life Span (ILS) in rats treated with *K. galanga* extract 73.27±10.51 with median value of 69.85% as compared to Vincristine group 53.84±11.94 with median 54.25%. Preliminary phytochemical tests of the candidate plant *K. galanga* indicated the presence of flavonoids, suggesting a prominent role for them in anticancer activity.

Key words: Anticancer, acute toxicity, cytotoxicity, flavonoids

INTRODUCTION

Cancer is one of the major causes of death worldwide. It is estimated that 12.5% of the population die due to cancer (WHO, 2004). Malignant disease is widely prevalent and, in the West, almost a third of the population develops cancer at some time during their life. It is second only to cardiovascular disease as the cause of death. Although the mortality of cancer is high, many advances have been made both in terms of treatment and in understanding the biology of the disease at the molecular level (Doll and Peto, 2003). Natural products have been always a rich

source of new chemical entities in the field of medicine and pharmacy. In the field of oncology, significant numbers of commercialized drugs have been obtained from natural sources, either by structural modification or by semisynthetic preparation. The search for improved cytotoxic agents is important in the discovery of modern anti-cancer drug (Nobili *et al*., 2009).

In recent years, the development of new anticancer drug has become a key issue because of the reality that cancer cells which are resistant to current chemotherapy will eventually dominate the cell population and cause the mortality. Furthermore, herbal medicines as alternative cancer therapy have attracted a great deal of recent attention due to their low toxicity and costs (Kang *et al*., 2005). Especially, clinically relevant anticancer drugs such as Taxol, Camptothecin, Vinblastin and Vincristine are well known phytochemicals which have anticancer property. Conventional anticancer drugs are associated with toxicity and worsen the quality of life. The extent of morbidity and mortality associated with metastatic conditions indicates clearly an urgent need for the discovery of new agents with higher clinical efficacy (Lee *et al*., 2003). Present knowledge on chemoprevention of cancer has revealed the presence of a diverse array of naturally occurring bioactive compounds, that inhibit the multistep process of carcinogenesis (Singh *et al*., 2006). Bioflavonoids, are a heterogeneous group of low molecular weight compounds have attracted attention in recent years due to their role as potentially important supplementary anticancer agents (Elangovan *et al*., 1994). Induction of apoptosis in tumor cells is considered very useful in the management and therapy as well as the prevention of cancer. Apart from physiological stimuli there are exogenous factors which can contribute to induction apoptosis. A wide variety of natural substances have been recognized to have the ability to induce apoptosis in various tumour cells of human origin (Taraphdar *et al*., 2001).

Flavonoids are the most abundant polyphenols present in the human diet in vegetables, fruits and plant-derived beverages and are the main components of many herbal products. Epidemiological and animal studies have suggested that a high intake of flavonoids may be beneficial to health and the lack of toxicity increases public interest in alternative medicine (Shuzhong *et al*., 2004). Hence, a study was carried out to screen the alcoholic extract of four indigenous plants for anticancer activity and identify the phytochemical constituents possibly responsible for the anticancer activity.

MATERIALS AND METHODS

The plants Clerodendrum viscosum (*Verbenaceaere*) Linn., Jatropha curcus (*Euphorbiaceae*) and *Lens culinaris* (Fabaceae) Linn. were collected from the tropical forests of Niligiris District, Tamilnadu, India. The *Kaempferia galanga* plant was collected from Kottiyur Reserve forest and compared with the herbarium specimen preserved at Botanical Survey of India, Coimbatore and authenticated by botanist Dr. V.S. Ramachandran, Reader, Department of Botany, Bharahtiar University, Coimbatore. The leaves of the plants *C. viscosum*, *J. curcus* and *L. culinaris* and the tubers of *K. galanga* were washed thoroughly with tap water, dried at room temperature in shade for 15 days and powdered. Thirty grams of powder was packed in the thimble of 100 mL soxhlet and extracted by percolation with 90% ethanol for 24 h. The extract was concentrated and dried at room temperature in a desiccator.

Brine shrimp assay: The cytotoxic activity of the extracts was evaluated by brine shrimp lethality assay according to the procedure of Solis *et al*. (1993). Brine shrimp (*Artemia salina*) eggs were obtained from Natural Remedies Pvt. Ltd. Bangalore, India. The eggs were transported and stored

in an airtight container at room temperature over a period of one month before hatching. The hatching chamber was fabricated as per the design used by Meyer *et al.* (1982). The eggs were hatched in artificial seawater prepared by dissolving 10 g of marine salt and 100 mg of sodium bicarbonate in 500 mL of water at room temperature and incubated for 48 h. Ten naupilii were pipetted out into glass vials of 10 mL capacity, the test solutions were added serially (10-150 μg mL^{-1}) and volume made up to 10 mL. A stock solution of the extract was prepared by dissolving 100 mg in 100 mL of artificial sea water (1 mg or 1000 μg mL^{-1}). Serial dilutions were prepared with artificial sea water to obtain a concentration of 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 110, 120, 130, 140 and 150 μg mL^{-1} solutions, respectively. Similar serial dilutions were prepared for vincristine (10-150 μg mL^{-1}). Equal volumes of artificial saline were used as control. Three replicates were conducted at each dose level and the average mortality rate was evaluated. The glass containers containing the nauplii in the presence of the extracts were incubated for 24 h at 25°C. The vials were then examined under a microscope and the proportion of viable cells and dead cells (non-motile nauplii) were manually enumerated. The concentration vs. percentage mortality rate curves of the various extracts and vincristine were compared with those of the control.

Screening for antitumor activity using potato disc inhibition assay: The antitumor activity of the extracts was evaluated by potato disc inhibition assay. Potato discs of 10 mm diameter and 0.5 mm thickness were sliced aseptically. *Agrobacterium tumifaciens* was cultured in Yeast Extract Media for 2 days at 28°C prior to inoculation. The bacterial culture was then inoculated onto the potato disc aseptically. Each disc was placed on the surface of 50% agar media and incubated at 28°C in a 24 well culture plate. The test samples consisted of 1 mL of extract; 1 mL of bacterial suspension and 0.25 mL of water. The control solution consisted of 1.25 mL of water and 1 mL of bacterial suspension. An aliquot of 50 μL of the mix of the appropriate extract/water/bacteria was placed and incubated at 25°C for 12 days. On day 12, the discs were stained with Lugol's reagent (I_2 KI; 5% I_2 plus 10% KI in distilled water) which stained the starch in the potato tissue a dark blue to dark brown color. However, the tumors produced by *A. tumifaciens* do not take up the stain and appear creamy to orange (McLaughlin, 1991). The stained potato disks were viewed under a (10 x) dissecting microscope and the numbers of tumors were counted. All the experiments were carried out in triplicate.

Screening for antimitotic activity using onion root method: The antimitotic activity was screened by onion root inhibition assay (Rai *et al.*, 2007). Onion bulbs were placed over beakers filled with tap water for two days till roots developed actively. The germinated root tips were excised and placed in a test tube and incubated for three h at room temperature in the presence of test and control solutions, respectively. The root tips were macerated and placed in a fixative solution of ethanol and 45% acetic acid (3:1) for 12 h. Then the root tips were hydrolyzed with hydrochloric acid (1 N) for 15 min at 60°C and placed on a clean glass slide. A drop of acetocarmine stain was added and the slide was heated gently until the root tips showed red discoloration. The cells were observed under (100 x) for different stages of cell divisions. Approximately 100 cells were counted per slide. The average mitotic index of three root tips was determined for each extract concentration and the experiment was repeated thrice. The mitotic index was determined as the percentage of the ratio of actively dividing cells to the total number of cells observed. A graph of extract concentration versus percent inhibition was plotted and he concentration required for 50%

mitotic inhibition (ID_{50}) was determined by extrapolation. The percent inhibition of mitosis by the extract at various concentrations was determined and compared with that of control.

Screening for tumor regression activity using Dalton Lymphoma Ascitic cell line (DLA) induced tumor in mice: All the extracts were screened for anticancer activity, using Dalton Lymphoma Ascitic (DLA) cell line induced tumor in mice (Devi and Ganasoundari, 1995). Female Swiss albino mice weighing 17-18 g were housed in polypropylene cages, at room temperature (25-30°C) and supplied with 100 g of food pellets and unlimited drinking water. Sterile paddy husk was used as bedding material. The mice were obtained from an inbred colony maintained under controlled conditions of light (10:14 h light: dark), temperature (23±3°C) and humidity. The mice were grouped into seven groups of six each as follows: Control; vincristine (5 mg kg^{-1}); *K. galanga* extract (500 mg kg^{-1}); *C. viscosum* extract (100 mg kg^{-1}); *J. curcus* extract (100 mg kg^{-1}); *L. culnaris* extract (100 mg kg^{-1}). A volume of 0.5 mL of the diluted extract was administered orally at a dose of 100 mg kg^{-1}. Dalton's lymphoma ascitic cell lines were procured from Amala Cancer Research Institute Centre, Thrissur, Kerala, India and propagated for 5 passages, by intraperitoneal transplantation of ascitic fluid in Swiss albino mice. After 15 days of tumor transplantation, the ascetic fluid (2 mL) was aspirated. Two milliliter of the fluid was diluted to 5 mL with saline to obtain a concentration of 2×10° cells per mL. From this stock solution, 0.5 mL was inoculated into the peritoneal cavity of healthy mice. Tumor viability was determined by the trypan blue exclusion test and cells were counted using hemocytometer in accordance with a study by Gupta (2002). The mice were weighed on the day of tumor inoculation and subsequently once every three days. Vincristine (one dose of 5 mg mL^{-1}) was injected from day 2 till day 7 intraperitoneally. The extracts were administered PO from day 3 day till day 45 or till the animal survived whichever was earlier. The mice were weighed using single pan weighing balance on day zero (one day prior to inducing lymphoma). They were subsequently weighed on days 10, 15, 20, 25 and 40 post inoculation. The percentage change in body weight was determined and adjusted appropriately with respect to the weight on day 0 (Tibiri *et al*., 2007; Eckhardt *et al*., 1982).

The percentage increase in body weight was calculated as follows:

$$\% \text{ Increase in weight} = \frac{\text{Weight of animal on test day-Weight of animal on day 0}}{\text{Weight of animal on day 0}} \times 10$$

The percentage increase in body weight was compared with that of control groups using one way ANOVA test. The mean bodyweight of each was compared with that of the control group using one way ANOVA test. The effect of four plant extracts on the survival time and percentage increase in life span of tumor bearing mice was evaluated (Devi and Ganasoundari, 1995). The Mean and the Median Survival Time (MST) and percentage increase in life span (%ILS) were calculated for the different groups. The total number of days an animal survived from the day of tumor inoculation was counted. Subsequently the median and mean survival time was calculated for each group. The percentage increase in life span (% ILS) was calculated as follows:

$$\text{Increase in life span} = \frac{(T\text{-}C)}{C} \times 100$$

where, T = MST for each extract and C = MST for DLA control group.

Statistical analysis: All the tests were carried out in triplicates and results expressed as Mean±SEM calculated with Excel 2007. The mean data of each group was statistically compared with that of the control by using one way ANOVA followed by Dunnets test. A p value $p<0.05$ was considered as significant difference. Linear correlation was used to identify the linearity of concentration Vs percentage mortality of brine shrimps. The mean survival time was compared between all the groups by one way ANOVA using SPSS version 11.

RESULTS

The ethanolic extracts of all the four plants viz. *K. galanga, C. viscosum, J. curcus* and *L. culnaris* exhibited significant linear concentration dependent cytotoxic action (Table 1). Mortality of brine shrimp was observed after 24 h of administration with ethanolic extract of *K. galanga*. *K. galanga* extract demonstrated better brine shrimp mortality at a concentration of 120 μg mL^{-1} after 24 h of incubation. The LC_{50} value was lowest for *K. galanga* extract (684.2 μg mL^{-1}) against the value of LC_{50} 901; 866; 5436 μg mL^{-1} for *C. viscosum, J. curcus* and *L. culnaris*, respectively. Vincristine demonstrated the lowest LC_{50} (0.7 μg mL^{-1}).

The anti-tumor activity of different concentrations of ethanolic extracts of *K. galanga, C. viscosum, J. curcus* and *L. culinaris* was studied on the *Agrobacterium* induced tumors in Potato discs. The results of the study indicate that all the ethanolic extracts caused a significant reduction in mean number of tumors produced by the *Agrobacterium* (Table 2). The alcoholic extract of *K. galanga* exhibited significant ($p<0.001$) inhibition of *Agrobacterium* induced tumors compared to control in potato discs with an average tumor count of 15, 11, 8.0, 6.0 and 4.8 at concentrations of 10, 20, 30, 40 and 50 μg mL^{-1}, respectively.

The anti mitotic activities of different concentrations of extracts were studied by the onion root method of antimitotic assay (Table 3). The results of the study showed that vincristine at all concentrations caused the maximum inhibition of mitosis. Alcoholic extract of various plants showed the maximum inhibition of mitosis in a dose dependent manner.

Table 1: Cytotoxic effect by brine shrimp assay

	% Mortality after 24 h					
Conc. of extract	Control	Vincristine	*K. galanga*	*C. viscosum*	*J. curcus*	*L. culinaris*
LC_{50} (μg mL^{-1})	0	380	684	901	866	5436
Linearity (r^2)	0	0.97	0.94	0.87	0.89	0.35
95% CI	0	0	(602.6-795.8)	(746.8-1149)	(734-1066)	(3023-2977)

Table 2: Antitumor activity by *Agrobacterium* induced tumors in potato discs

	Tumor count (Mean±SEM)				
Treatment groups	10 μg mL^{-1}	20 μg mL^{-1}	30 μg mL^{-1}	40 μg mL^{-1}	50 μg mL^{-1}
Control	21.0±1.2	21.8±1.6	20.3±1.3	19.3±0.83	25.8±1.0
Standard drug (vincristine)	19.4±0.51*	16.8±0.83*	14.2±0.98*	8.5±1.0*	8.3±0.76*
K. galanga extract	15.0±0.8*	11.8±0.6*	8.0±0.89*	6.0±0.57*	4.8±0.40*
C. viscosum extract	22.2±0.48*	21.2±0.70*	19.8±0.98*	16.3±1.1*	12.5±1.17*
J. curcus extract	19.2±0.91*	17.5±1.4*	14.5±0.84*	12.5±0.43*	13.7±0.95*
L. culnaris extract	18.5±0.95*	15.33±0.4*	18.3±1.6*	14.3±0.84*	12.5±0.76*

*$p<0.001$ significant difference compared to negative control

Table 3: Antimitotic screening by onion root method

Drugs	Concentration (μg mL^{-1})	Mitotic index	% Inhibition
Control		100	
Vincristine	50	3	97
	100	0	100
K. galanga extract	50	20	80
	100	10	90
C. viscosum extract	50	60	40
	100	50	50
J. curcus extract	50	35	65
	100	25	75
L. culnaris extract	50	60	40
	100	40	60

Table 4: Antitumor screening by assessment of tumor related body weight changes in DLA tumor induced mice

	% Body weight gain (Mean±SEM)				
Treatment groups	Day 10	Day 15	Day 20	Day 25	Day 40
Normal control	0.5±0.04	1.4±0.4*	2.1±0.32*	2.7±0.31*	5.02±0.79*
Negative control	42.7±1.9[a]	44.8±2.2[a]	-100[a]	-100[a]	-100[a]
Standard drug (vincristine)	4.03±0.86*	6.6±0.8*	4.8±0.36*	0.6±21.9	-100[a]
K. galanga EtOH extract	4.4±0.98*	4.5±0.80*	1.6±0.93*	0±0.94*	35.5±20.4*
C. viscosum EtOH extract	22.5±3.73[a]*	22.9±3.50[a]*	37±28.3[a]*	36.6±28.4	56.5±27.6[a]
J. curcus EtOH extract	17.8±3.3[a*]	23.04±4.56[a*]	56±27.3	56.4±27.5	-100[a]
L. culnaris EtOH extract	14.2±4.6[a*]	19.4±3.16[a]*	58.7±26.5	58.7±26.5	-100[a]

*$p<0.001$ significant difference compared to negative control group (DLA inoculated control group). [a]$p<0.05$ significant difference compared to untreated control group

As shown in Table 4 the untreated control mice gained a substantial tumor related weight on the tenth day as compared to that on the first day. There was weight gain of 42.7% by day 10 and 44.8% by day 15. Treatment with vincristine (5 mg kg^{-1}) significantly ($p<0.001$) reduced the weight gain as compared to negative control animals by day-10 (4.03 g), day-15 (6.6 g) and day-20 (4.8 g). Treatment with *K. galanga* extract significantly ($p<0.001$) reduced the weight gain by day-10 (4.4 g), day-15 (4.5 g), day-20 (1.6 g) and day-40 (35.5 g) as compared to the negative control group. On day 25 there was no increase in weight. Ethanolic extracts of all the other plants significantly ($p<0.05$) reduced the weight gain by day 10 and day-15 as compared to negative control. Among all these extracts *K. galanga* demonstrated significant reduction of tumor related weight for all the periods (15, 20, 25 and 40) days as compared to control. A significant difference ($p<0.001$) in mean body weight was observed between different treatment groups and negative control animals. The results of tumor regression effect of ethanolic extracts of *K. galanga, C. viscosum, J. curcus* and *L. culnaris* are presented in Table 5. The average% ILS following vincristine treatment was 53.84±11.94% with a median of 54.25 (IQR 23.17-76.6). The average% ILS for *K. galanga* treatment was 73.27±10.51 with median value of 69.85 (IQR 53.82-95.0). There was a statistically significant ($p<0.001$) higher% ILS with *K. galanga* extract than those for the other three extracts. There was equipotent tumor regression action in *K. galanga* extract 18.5 g on day 10 and vincristine from day 10 (18.5 g) to day 25 (18.4). However, *K. galanga* extract demonstrated a better tumor regressive activity than the vincristine treatment group.

Table 5: Antitumor screeing by assessment of tumor regression in DLA tumor induced mice

	Body weight (Mean±SEM)					
Treatment groups	Day 1	Day 10	Day 15	Day 20	Day 25	Day 40
Control	17.7±0.15	17.80±0.18	17.9±0.18	18.1±0.15	18.1±0.15	18.6±0.05
Negative control	17.7±0.05	25.30±0.31	25.3±0.31	25.8±0.06	26.4±0.15	26.0±1.6
Vincristine	17.7±0.09	18.50±0.35*	18.9±0.10*	18.6±0.06*	18.4±0.39*	18.1±0.14*
K. galanga extract	17.7±0.09	18.50±0.26*	18.51±0.8*	17.9±0.15*	17.5±0.14*	17.4±0.18*
C. viscosum extract	18.3±0.04	22.40±0.69*	22.5±0.69*	22.5±0.74*	22.5±0.83*	22.4±1.00[a]
J. curcus extract	17.7±0.07	20.80±0.52*	21.7±0.75*	22.4±0.74*	22.9±0.84*	23.6±0.93
L. culnaris extract	17.6±0.05	20.15±0.8*	21.1±0.54*	21.3±0.47*	21.6±0.55*	22.1±0.64[a]

*p<0.001 significant difference compared to negative control (DLA inoculated control) (Two way ANOVA). [a]p<0.05 significant difference compared to negative control

Table 6: Antitumor screening by assessment of survival period of tumor bearing mice

	Survival time		% Increase of Life Span (ILS)	
Treatment group	Mean±SEM	Median (Interquartile range)	Mean	Median (Interquartile range)
DLA tumor bearing control	16.83±0.48	17.0 (15.75-18.0)	-	-
Vincristine (5 mg kg^{-1})	25.67±1.43*	26.5 (21.7-28.5)	53.83±11.94[a]	54.25 (23.17-76.6)
K. galanga	29.00±1.37*	28.0 (26.5-32.5)	73.27±10.51[a]	69.85 (53.82-95.0)
C. viscosum	28.67±5.08*	24.5 (18.0-42.25)	68.37±27.53[a]	46.7 (10.85-138.5)
J. curcas	22.83±3.87*	17.5 (16.0-34.5)	34.75±20.99[a]	10.0 (-5.67-100)
L. culnaris	20.83±2.15*	18.5 (16.75-27.25)	23.15±10.77[a]	6.5 (5.82-56.4)

*p<0.001 significantly different from the control animals. [a]p<0.001 significantly different from each other

The effect of four plant extracts on the survival of tumor bearing mice is shown in Table 6. The Mean Survival Time (MST) for tumor bearing control mice was 16.8±1.2 days. There was significant ($p<0.05$) increase in survival following the administration of *K. galanga* extract. Treatment with *K. galanga* extract significantly increased the life span (73% ILS) with a mean MST of 29.0±1.4 and a median survival of 69.8 days. The MST of tumor bearing mice was 28.6±12.4, 22.8±9.4 and 20.8±5.2days, for *C. viscosum, J. curcus* and *L. culnaris* extracts respectively. The MST of standard drug vincristine group was 25.6±3.5 days.

Based on *In vitro* studies like potato disc method and onion root inhibition studies the ethanolic extract of *K. galanga* was found to possess significant antitumor activity. *In vivo* studies on DLA induced tumor in mice indicated that the extract of *K. galanga* had significantly higher tumor regression activity, increased survival time and increase in life span. Hence the plant *K. galanga* was chosen as candidate plant for more detailed pharmacognostical and phytochemical studies.

DISCUSSION

In the present study, the anti-tumor activity of different concentrations of ethanolic extracts of *K. galanga, C. viscosum, J. curcus* and *L. culnaris* was evaluated. Ethanolic extracts demonstrated strong inhibitory effect on the growth of cancer cells in DLA tumor bearing mice. Based on *in vitro* studies like brine shrimp lethality assay, potato disc method and onion root tip inhibition studies, the ethanolic extract of *K. galanga* was found to possess significantly better anticancer activity than the other extracts. *In vivo* studies on DLA tumor bearing mice revealed that *K. galanga* extracts exhibited remarkable tumor regression and body weight reduction and

prolonged the survival time. Total content of phenolic compounds and flavonoids was also estimated by spectrophotometric method. Similar *in vitro* cytotoxicity study was conducted on $HepG_2$ cell line with increasing concentrations and the apoptotic activity was determined by Dhanasekaran and Ganapathy (2011). There are several *in vitro* studies and rodent *in vivo* studies suggesting that certain herbs and spices may have a chemopreventive effect in the early (initiating) stages of cancer. Herbs may act through several mechanisms to provide protection against cancer (Surh, 2002). Certain phytochemicals from herbs or herb extracts have been shown to inhibit one or more of the stages of the cancer process (i.e., initiation, promotion, growth and metastasis) (Surh *et al*., 1998).

The brine shrimp lethality bioassay is efficient and rapid with inexpensive tests that require relatively small amounts of samples. Meyer *et al*. (1982) have successively employed brine shrimp assay for *in vitro* lethality bioassay-guided fractionation of active cytotoxic and antitumor agents such as trilobacin from the bark of *Asimina triloba*. Pervin *et al*. (2006) purified lectins from the rootstock of Pondweeds (*Potamogeton nodosus* Poir)and proved their cytotoxic effect by brine shrimp (*Artemia salina* L.) lethality bioassay. According to their study, the LD_{50} of purified lectins PNL-1, PNL-2, PNL-3, PNL-4, PNL-5 and PNL-6 was 10.76, 7.03, 17.25, 10.52, 19.60 and 20.1 μg mL^{-1}, respectively (Pervin *et al*., 2006). Anderson *et al*. (2007) compared simple bench-top bioassays involving brine shrimp lethality, *Lemna* frond proliferation and the inhibition of crown gall tumors on potato discs, as well as the human tumor cell lines (A-549 lung carcinoma, MCF-7 breast carcinoma and HT-29 colon adenocarcinoma) for their accuracy unestum ting the active antitumor activity of agents supplied by the National Cancer Institute. They reported that the potato disc assay was the best model that demonstrated excellent correlation to *in vivo* activity ($p = 0.008$). The anti mitotic activities of different concentrations of extracts were determined by the onion root method of antimitotic assay. All the alcoholic extracts showed inhibition mitotic in a dose dependent manner compared to the control. Vincristine inhibited mitosis at all concentrations. A reduction in mitotic activity could be due to the inhibition of DNA synthesis which is considered as one of the major prerequisites for a cell to divide. Nithyameenakshi *et al*. (2006) reported difenoconazole depressed mitotic index by 4.305% over untreated control at 12 h exposure period. The extent of chromosomal abnormalities has direct relationship with the concentration of the active ingredients and treatment time.

In vivo studies on DLA induced tumor in mice indicated that the extract of *K. galanga* had greater tumor regression activity and increased the survival time and life span of the mice. Hence the plant *K. galanga* was chosen as candidate plant for more detailed pharmacognostical and phytochemical studies. Ethanolic extracts of all the other plants significantly ($p<0.05$) reduced the weight gain by day 10 and day 15 as compared to negative control. Among all these extracts *K. galanga* demonstrated the best reduction in tumor related body weight during the observation periods (day 15, 20, 25 and 40) as compared to control. An enhancement of life span by 25% or more over that of control was considered as an effective antitumor response (Green *et al*., 1983). Indian medicinal herbs are rich sources of potential novel anticancer agents. Flavonoids are believed to possess cytotoxic action against fast growing cells like cancer cells and not to affect the normal slow growing cells. Plant extracts also have minimum or no side effects when compared to the cytotoxic chemotherapeutics which may themselves be carcinogenic. Flavonoids are usually present in plants as mixtures that include colored anthocyanins and colorless flavones or flavonols. Estimation by standard procedures developed by Harborne (1998) shows the presence of different flavonoids, phenolic compound, tannins, alkaloids and saponins.

CONCLUSION

Cytotoxic activity was determined for the alcoholic extracts of four plants *Jatropha curcus, Clerodendrum viscosum, Lens culnaris* and *Kaempferia galanga* using *in vitro* and *in vivo* studies. *K. galanga* seemed to exhibit significant cytotoxic and antitumor activity compared to the other plant extracts. They may represent a new line of antitumor agents for prevention and treatment of cancer and also serve as a potential source of chemoprotective agent (s). Systematic and rigorous investigation of their active ingredients and mechanisms of action are crucial to provide evidence ad rationale for their efficacy and for transforming traditional herbal practice into evidence-based medicine. Further studies on the mechanism of cell death are needed to provide more convincing evidence.

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