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Anticancer Activity of Leaves of *Clerodendron Serratum* Spreng

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ABSTRACT

Clerodendron serratum spreng is very much effective in preventing Dalton's Ascetic Lymphoma cell in mice which was confirmed by evaluating the hematological parameters. Peritoneal cell count, solid tumors volume, body weight and histopathological studies. This holds great promise for future research in human beings. The anticancer properties of *Clerodendron serratum* spreng will provide useful information in the possible application in cancer prevention and cancer therapy. The treatment with ethanolic and aqueous extracts of *Clerodendron serratum* spreng significantly altered all the parameters, near to normal. Maximum alteration of parameters occurred in the group treated with the aqueous extract at the dose of 300 mg/kg/day.

Keywords: Anticancer, *Clerodendron serratum* spreng, Verbenaceae, Soxhlet apparatus, Swiss albino mice

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INTRODUCTION

The isolation of secondary plant metabolites begins with the selection of a plant the most critical aspect of the project in order to locate a plant C*lerodendron serratum.*The aim of the present study is to evaluate the plant for pharmacognostical, phytochemical and pharmacological activity. The literature survey clearly shows that the "*Clerodendron serratum* spreng" has been traditionally used as hepatoprotective, antibiotic, antioxidant, diuretic and astringent. Based on these uses, the present study was aimed to investigate the anti cancer activity of the leaves of" *Clerodendron serratum* spreng*".*

The plant *Clerodendron serratum* spreng is widely distributed all over India and especially in the Himalaya region, Tamilnadu, Madhya Pradesh, peninsular region etc. For this project work, the plant was collected from the Yercaud hills Salem, Tamilnadu. The collected leaves were shade dried well and powdered by home grinding mill. Then the powder was passed through sieve no-40, and stored in air tight container.

Taxonomy

Plant name : *Clerodendron serratum* spreng.

Family : Verbenaceae

The roots and leaves of bharangi have great medicinal value. The plant is useful both, internally as well as externally. The leaves are useful as an external application for cephalalgia and ophthalmia. The pulp of the leaves applied externally, mitigates the glandular swellings and hastens the wound healing. The juice of its leaves is applied on the lesions in erysipelas. The root paste applied on the forehead alleviates headache[1].

Morphological character

The botanical name of bharngi is *Clerodendrum serratum* and it belongs to family Verbenaceae. From the bark the sapogenin mixture contains three major triterpenoid constituent's oleonolic acid, queretaroic acid and serratagenic acid. The root bark yields a glycoside material, phenolic in natures. D – Mannitol is isolated from the bark with a yield of 10.9 %. The powdered stem contains D- mannitol, D- glucoside of sitosterol, sitosterol and cetyl alcohol. Alcoholic extract and saponin isolated from root bark caused release of histamine from lung tissue.[1, 2]

MATERIALS AND METHODS

The required samples (Dalton's Ascetic Lymphoma cell) of different organs were cut and removed from the plant and fixed in FAA (Formalin - 5 ml + acetic acid - 5ml + 70% Ethyl alcohol - 90ml). After 24 hrs of fixing, the specimens were dehydrated with graded series of

tertiary - butyl alcohol as per the schedule given by Sass, 1940.[3] Infiltrations of the specimens were carried out by gradual addition of paraffin wax (melting point 58 – 60^{0}C) until TBA solution attained supersaturation. The specimens were casted into paraffin blocks.

a) Collection: The required samples of different organs were cut and removed from the plant and fixed in FAA (Formalin - 5 ml + acetic acid - 5ml + 70% Ethyl alcohol - 90ml). After 24 hrs of fixing, the specimens were dehydrated with graded series of tertiary - butyl alcohol as per the schedule given by Sass, 1940.[(3)] Infiltrations of the specimens were carried out by gradual addition of paraffin wax (melting point 58 – 60^{0}C) until TBA solution attained supersaturation. The specimens were casted into paraffin blocks.

b) Sectioning: The paraffin embedded specimens were sectioned with the help of Rotary Microtome. The thicknesses of the sections were 10-12 μm.

The sections were stained with Toluidine blue.[4] Since Toluidine blue is a polychromatic stain, the staining results were remarkably good; and some Cytochemical reactions were also obtained. The dye rendered pink color to the cellulose walls, blue to the lignified cells, dark green to suberin, violet to the mucilage, blue to the protein bodies etc. Wherever necessary sections were also stained with safranin and Fast – green and IKI (for Starch). For studying, the microscopy (sections taken parallel to the surface of stem) as well as clearing of root with 5% sodium hydroxide was prepared. Glycerine mounted temporary preparations were made for macerated/ cleared materials. Powdered materials of different parts were cleared with NaOH and mounted in glycerine medium after staining.

For the study of crystals, starch grains and lignified cells, polarized light was employed. Since these structures have bi-refringent property, under polarized light they appear bright against dark background.

Preparation of various extracts of leaves of *Clerodendron serratum* spreng

The leaves of *Clerodendron serratum* spreng were collected and air dried under shade and then coarsely powdered with the help of mechanical grinder. The powder was passed through sieve no.10 and stored in an airtight container for further studies. 150 gms of powdered material was evenly packed in the Soxhlet apparatus. It was then defatted with petroleum ether and extracted with ethanol. The aqueous extraction was carried out by cold maceration process.

Requirements:

1. Shade dried coarse powder of leaves of *Clerodendron serratum* spreng.
2. Soxhlet apparatus.

Solvents used:

Petroleum ether.

Alcohol 70% v/v.

Distilled water: chloroform (90:10)[5,9]

After completion of the extraction, the crude ethanolic and aqueous extracts were separately filtered and the solvent was removed by evaporation to dryness on a water bath. The dried extracts were stored in desiccators to remove excessive moisture.

Animals

Swiss Albino mice (20-25gm) of either sex and of approximately the same age were procured from the listed supplier Mr. Venkateswara Enterprises, Bangalore and used for the study. They were housed in polypropylene cages and fed with standard rodent pellet diet (Hindustan Lever Limited Bangalore) and water *ad libitum*. The animals were exposed to alternate cycle of 12 hrs of darkness and light each. Before each test, the animals were fasted for at least 12 hrs; the experimental protocols were subjected to the securitization of the Institutional Animals Ethical Committee (P. Cog/14/2007) and were cleared by the same.

Procedure

The substance is tested using a stepwise procedure, each step using three animals, all animals of single sex (normally female mice). Absence or presence of mortality of the animal doses at one step will determine the next step. The ethanolic and aqueous extract of leaves of *Clerodendron serratum* spreng were screened for acute toxicity study by OECD guideline[7].

EVALUATION OF ANTICANCER ACTIVITY

Experimental Setup:

The animals (Swiss albino mice weighing 20-25g) were divided into 5 groups of 6 animals each. Animals were fed with basal diet and water throughout the experiment. Animals of Group II to Group V were inoculated with $1x10^6$ DAL cells administered by intraperitoneal route[6].

Group I - Normal Control
Control animals received normal Saline

Group II - Tumour Control
Animals inoculated with $1x10^6$ cells per mouse intraperitonearly

Group III - Standard Group
Animals were injected with 5-fluorouracil (200mg/kg) [8]

Group IV - Test Group I

Animals were administered with ethanolic extract of *Clerodendron serratum* spreng (300 mg/kg) orally.

Group V - Test Group II

Animals were administrated with aqueous extract of *Clerodendron serratum* spreng (300 mg/kg) orally.

Ethanolic and Aqueous extracts

All the treatments were given for 9 days. On the 14th day, all the animals were sacrificed under ether anesthesia and blood was drawn by retro orbital plexus method. WBC count, RBC count, hemoglobin, protein and packed cell volume were determined. Cells smear was prepared in slide and stained with Lieshman Stain Solution**.**[4] Red blood cells (RBC), White blood cells (WBC) and Haemoglobin (Hb) were estimated with the help of hematology analyzer (Medonic CA620, Boule, Sweden). The RBC and WBC were expressed as 10^6/mm^3 and 10^3/mm^3 of blood and Hb as g/dl of blood.

Effect of Ethanolic and Aqueous extracts of leaves of *Clerodendron serratum* spreng on solid tumour

To determine the effect of ethanolic and aqueous extracts of Leaves of *Clerodendron serratum* spreng on solid tumor, all the animals were injected with 1 x 10^6 cells/ mouse in phosphate buffered saline into the right hind limb of all the animals subcutaneously. All the treatments were started from the next day of inoculation and were continued for five alternative days. The measurement of tumor radii was taken from 11th day of tumor induction and was repeated on every 5th day for a period of 30 days. The volume on mass calculated from the formula $V=4/3\pi r^2$ where 'r' is the mean of r_1 and r_2 which are the two independent radii of the tumor mass.

Effect of Ethanolic and Aqueous extracts of leaves of *Clerodendron serratum* spreng on peritoneal cells in normal mice

One group of animals was treated with aqueous extract at a dose of 500mg/kg/day, leaves of *Clerodendron serratum* spreng once for a single day and the second group received the same treatment for two consecutive days. Similar treatment was given to other groups with used as control. Peritoneal exudates of ethanolic and aqueous extract groups were collected after 24hrs and 48 hr of treatment by repeated intraperitoneal with normal saline (0.9 % w/v) and the cells were counted in each of the treated groups under WBC newvbauer's chamber and compared with those of normal control[10,11].

Ethanolic and Aqueous extracts of leaves of *Clerodendron serratum* spreng on body weight

Four groups of six mice each were transplanted intraperitoneally with 1 x 10^6 Dalton's ascetics' tumour cells. After 24hr, the first and second groups were orally treated with alcoholic and aqueous extracts of *Clerodendron serratum* spreng. The third group, serving as the control, received normal saline (0.9%w/v). Treatments were continued for 9 days. Body weights were recorded every 5th day till 40 days of treatment.[12,13]

Histopathology report

Cytology Report of Gastric Aspirate

Group 1 –Normal; received about 1ml of watery fluid

Group 2 – Tumor control; received about 1ml of watery fluid.

Total cell count:	100 cells/cumm
Differential cell count :	Benign epithelial cells: 100%
	Malignant epithelial cells: 0%
	Inflammatory cells: 0%

Smear cytology**:** Smears show a few dispersed epithelial cells of the gastric mucosa. The cells show moderate cytoplasm and round to oval nuclei. There are no malignant cells.

Group 3 – Standard control:	Received about 1ml of watery fluid.
Total cell count:	50 cells/cum m
Differential cell count :	Benign epithelial cells: 50%
	Malignant epithelial cell: 0%
	Inflammatory cells: 50%

Smear cytology**:** Smears show a few dispersed epithelial cells of the gastric mucosa. The cells show moderate cytoplasm and round to oval nuclei. There are no malignant cells. A few scattered lymphocytes are seen.

Group 4 –Test group (A) Ethanolic extract; received about 1ml of watery fluid.

Total cell count:	5000 cells/cumm
Differential cell count :	Benign epithelial cells: 10%
	Malignant epithelial cell: 90%
	Inflammatory cells: 0%

Smear cytology: Smears show a high cellular yield with numerous malignant cells. The cells show large pleomorphic and hyperchromatic nuclei with moderate cytoplasm (adenocarcinoma cells).

Group 5 –Test group (B) Aqueous extract; received about 1ml of watery fluid.

Total cell count: 1400 cells/cumm

Differential cell count : Benign epithelial cells: 35%

Malignant epithelial cells: 60%

Inflammatory cells: 05%

Smear cytology: Smears show a moderate cellular yield with numerous malignant cells. The cells show large pleomorphic and hyperchromatic nuclei with moderate cytoplasm (adenocarcinoma cells). There are a few benign epithelial cells and occasional lymphocytes.

RESULT AND DISCUSSION

Data showing the ash values of extract of dried leaves of *Clerodendron serratum* spreng

The dried leaves of *Clerodendron serratum* spreng on Ash values increasing polarity by Crucible Apparatus. The percentage yield of the dried leaves of *Clerodendron serratum* spreng was found to be 7.33%w/w, 7.03%w/w, 0.30%w/w, total ash, water soluble, acid in soluble respectively.

Data showing the solid tumour volume of dried leaves of *Clerodendron serratum* spreng

The extract of *Clerodendron serratum* spreng also showed remarkable reduction in solid tumour size. The results are shown in **(Table 1.).** Tumour bearing in mice was shows significantly increased tumorur volume viz. 15^{th}, 20^{th}, 25^{th}, & 30^{th}day respectively.

Table 1. Data showing the solid tumour volume of dried leaves of *Clerodendron serratum* spreng.

Design of treatment	Solid tumorur volume (ml)			
	15^{th}days	20^{th}days	25^{th}days	30^{th}days
Tumorur control	5.5±0.22*	6.0±0.25*	7.0±0.28*	8.5±0.3*
5- fluorouracil(20mg/kg)	3.5±0.22**	3.4±0.21**	3.0±0.20**	2.5±0.2**
Eth(300mg/kg/day,p.o)	5.0±0.22**	5.4±0.21**	5.8±0.22**	6.5±0.25**
Aqu (300mg/kg/day,p.o)	4.5±0.22**	5.0±0.21**	5.5±0.22**	6.5±0.25**

Values were expressed as mean ± SEM, n=6 in each group.
** P<0.001 Compared to respective Cancer control group,
*P<0.05 Compare to Normal control group,
Data were analyzed by using one way ANOVAs test

The tumorur volume of control animals on 30^{th}day was 8.5±0.3ml, where as it was 6.5±0.25 ml (p<0.001) for ethanolic (300mg/kg/day, p.o) and6.5±0.25 ml (p<0.001) for aqueous (300) extract treated animals. The standard group animal were treated with 5-fluorouracil shown tumour volume of 2.5±0.2 ml (p<0.001). The maximum inhibition of tumour volume was produced by the aqueous extract at a dose of 300mg/kg/day p.o and may be due to cytotoxic effect on DAL

cells. The diminution of tumour size indicates the antitumour activity of *Clerodendron serratum* spreng.

Data showing the Haematological parameters of dried leaves of *Clerodendron serratum* spreng.

The effect on ethanolic and aqueous extracts of *Clerodendron serratum* spreng on hematological parameters of tumour bearing mice is shown in **Table 2.** Hematological parameters of tumour bearing mice on day 14 showed significant changes when compared to the normal mice. The tumour bearing mice shows decreased level of Haemoglobin, Red Blood Cells, Lymphocytes and increase level of White Blood cells, Total Protein, Packed Cell Volume and Neutrophils. The treatment with ethanolic and aqueous extracts of *Clerodendron serratum* spreng significantly altered all the parameters, near to normal. Maximum alteration of parameters occurred in the group treated with the aqueous extract at the dose of 300 mg/kg/day p.o. Fourteen days after transplantation, Ethanolic and Aqueous extract treated groups were able to reverse the changes in the haematological parameters consequent to tumour inoculation.

Table.2. Data showing the Hematological parameters of dried leaves of *Clerodendron serratum* spreng.

Treatment/ Dose	Hb (g/dl)	RBC (million /mm^3)	WBC (million /mm^3)	Protein g %	PCV (mm)	Neutrophils%	Lymphocytes %	Monocytes %
Normal	10.1 ±0.10	6.07 ±0.06	5.3 ± 0.03	8.21 ± 0.06	16.5 ± 0.42	30.83 ± 0.60	78.5 ± 0.42	11.3 ± 0.16
Tumour Control	9.2 ± 0.09*	4.62 ± 0.02*	49.2 ± 0.20*	26.12 ± 0.6*	31.5 ± 0.42*	68.83 ± 0.60*	35.2 ± 0.62*	9.6 ± 0.16*
Tumour+5-FU(20mg/kg)	11.2 ± 0.02**	5.02 ± 0.02**	12.8± 0.04**	20.42 ± 0.56**	19.5 ± 0.42**	31.83 ± 0.47**	73.2 ± 0.42**	18.1 ± 0.21**
Ethanolic (300mg/kg)	12.4 ± 0.20**	5.92 ± 0.12**	18.2 ± 0.22**	22.2 ± 0.62**	24.4 ± 0.42**	42.16 ± 0.60**	79.1 ± 0.68**	18.0 ± 0.22**
Aqueous (300mg/kg)	14.6 ± 0.12**	5.16 ± 0.14**	22.12 ± 0.04**	24.6 ± 0.02**	21.3 ± 0.03**	38.0 ± 1.78**	85.7 ± 0.42**	11.0 ± 0.33**

Values were expressed as mean ± SEM, n=6 in each group, ** P<0.001 Compared to respective Cancer control group

*P<0.05 Compare to Normal control group, Data were analyzed by using one way Anova test

Data showing the Body weight of mice of dried leaves of *Clerodendron serratum* spreng

The effect of extract of *Clerodendron serratum* spreng on average body weight of tumour bearing mice was also studied. The results are shown on **Table.3.** The average weight gain of tumour bearing mice on 21th days was 27.83±79. Normal animal shown a weight gain of 31.83 ± on 35th day. The animals treated with 5-fluorouracil shown an average body weight of 32.83 ±

0.47 on 35th day. The animals treated with the ethanolic and aqueous extract of *Clerodendron serratum* spreng shown average body weight of 37.33± 0.33 (P<0.001 Vs standard) and 35.83 ± 0.30 (P<0.001Vs standard) respectively on the 35th day.

Table.3. Data showing the Body weight of mice of dried leaves of *Clerodendron serratum* spreng.

Group No.	Group	Dose/ Route	7th Day	14th Day	21th Day	28th Day	35th Day
1	Normal control	-	22.0 ± 0.77	23.0 ±0.51	24.16 ±0.54	27.66 ± 0.66	31.83 ± 0.83
2	Tumour control	-	50.16 ± 0.65*	40.33 ± 0.76*	27.83 ± 0.79*	-	-
3	Tumour +5-FU(20mg/kg)	20mg/kg/ day.i.p	23.33 ± 0.61**	24.5 ± 0.34**	26.83 ± 0.60**	29.33 ± 0.66**	32.83 ± 0.47**
4	Tumour + Ethanolic extr.	300mg/kg/ day p.o	25.0 ± 0.25***	30.66 ± 0.33***	32.3 ± 0.66**	34.33 ± 0.33**	37.33 ± 0.33***
5	Tumour + Aqueous extr.	300mg/kg/ day p.o	24.33 ± 0.33**	29.5 ± 0.76**	30.5 ± 0.88**	31.0 ± 0.25***	35.83 ± 0.30***

Values were expressed as mean ± SEM, n=6 in each group, ***P<0.01, ** P<0.001 *P<0.05, Data were analyzed by using one way Anova test

CONCLUSION

Recent studies on tumor inhibitory compounds of plant origin have yielded an impressive array of research on medicinal plant. The efficacy of *Clerodendron serratum* spreng against Dalton's Ascitic Lymphoma described in the present investigation offer the potential for reaching on understanding of anticancer potency.

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