



**ANTICANCER ACTIVITY OF THE WHOLE PLANT OF ETHANOL
EXTRACT OF *POLYGALA CHINENSIS* L. (POLYGALACEAE)**

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ABSTRACT

The present study aims to evaluate the antitumor activity of ethanol extract of whole plant of *Polygala chinensis* L. on DAL model in Swiss Albino mice. Evaluation of the antitumor effect of ethanol extract of whole plant of *Polygala chinensis* on tumor growth and hosts survival time was made by the study of the following parameters: tumor volume, viable and non viable cell count and life span of host. The results showed decrease in tumor volume and cell viability. Hematological studies revealed that, the Hb count decreased in DAL treated mice, whereas it was induced by the drug treated animals and showed an increase in Hb near to normal levels. The results suggested that the extracts of whole plant of *Polygala chinensis* exhibited significant antitumor activity on DAL bearing mice.

Key words: *Polygala chinensis*, Tumor volume, Life span.

INTRODUCTION

In recent times, medicinal plants occupy an important position for being the paramount sources of drug discovery.¹ Plants have been indispensable in treating diverse forms of diseases including cancer. According to World Health Organisation, 80% of the people living in the rural areas depend on medicinal plants as primary health care system. These practices are solely based on the knowledge of traditional use of medicinal plants. Natural products are formulated to generate different types of effective drugs to enhance anticancer activities. Proper understanding of the complex synergistic interaction of various constituents of anticancer herbs, would help in formulating the design to attack the cancerous cells without harming the normal cells of the body^{2,3}.

Cancer is a dreadful disease characterized by the irregular proliferation of the cells. As a cell progress as from normal to cancerous, the biological imperative to survive and perpetuate drives fundamental changes in cells behavior. So the actual cause of the disease in different sections is still to be explored clearly. Cancer is thus, a class of diseases, classified by the type of cell that is initially affected. Today's global scenario indicates that breast cancer and colorectal cancer is the most prominent cancer in case of woman and man. To combat cancer United States National Cancer Institute has undergone 2069 anticancer clinical trials, in which over 150 drug combinations have been successfully recorded against cancer. The search for this cancer drug discovery from natural sources began with the investigations done by

Hartwell and his co-workers in the late 1960's with the application of Podophyllotoxin and its derivatives from the plant *Podophyllum peltatum*. Further discoveries lead to isolate anticancer compounds from plants like *Catharanthus roseus*, *Camptotheca acuminata* and *Taxus brevifolia*. Vincristine, Vinblastine, Camptothecin and Tawol are the established potential anticancer agents derived from these plants, which are found to be effective against various types of cancer⁴.

Polygala was traditionally used by Americans to treat snake bites⁵ and as an expectorant to treat cough and bronchitis. *Polygala* is considered as a powerful tonic herb⁶ that can help to develop the mind and aid in creative thinking. However, inspite of traditional use, pharmacology of its whole parts has not yet been explored scientifically. So far no reports are available in anticancer activity of the ethanol extracts of whole plants of *Polygala chinensis* against *in vivo* Dalton Ascites Lymphoma (DAL) tumor model.

Polygala chinensis L. belongs to Polygalaceae family. It is commonly known as "Siriyanangai". Taking into consideration of the medicinal importance of *Polygala chinensis*, the ethanolic extract of whole plant of *Polygala chinensis* were analyzed for the first time using GC-MS. This work will help to identify the compounds of therapeutic value.

EXPERIMENTAL

Materials and methods

Collection

The whole plants of *Polygala chinensis* L. were collected in the month of February and March, 2010, from the Vadavalli, Coimbatore District, Tamil Nadu. The plant specimen were identified with the help of local flora and authenticated in Botanical survey of India, Southern Circle, Coimbatore, Tamil Nadu, India. A voucher specimen was deposited in Ethnopharmacology Unit, Research Department of Botany, V.O. Chidambaram College, Tuticorin, Tamil Nadu.

Preparation of plant extract for anticancer activity

The whole plants of *Polygala chinensis* were cut into small pieces, washed dried at room temperature; the dried whole plant were powdered in a Wiley mill. 100 g of powdered whole plant were separately packed in a Soxhlet apparatus and extracted with ethanol. The ethanol extracts were concentrated in a rotary evaporator. The concentrated ethanol extracts of whole plant were used for anticancer activity.

Animals

Healthy male adult Swiss Albino mice (20-25 g) were used for the study. The animals were housed in microlon boxes in a controlled environment (temperature 25 ± 20 c) and 12 hr dark/eight cycle) with standard laboratory diet (Sai Durga Feeds and Foods, Bangalore) and water *ad libitum*. The mice well segregated based on their gender and quarantined for 15 days before the commencement of the experiment. They were fed on healthy diet and maintained in hygienic environment in our animal house.

Tumor cells

Dalton Ascites Lymphoma (DAL) tumor model cells were obtained from Division of Oncology Department of Biotechnology, Tamil Nadu, Veterinary and Animal Husbandary, Chennai, Tamil Nadu, India. The DAL cells were maintained *in vivo* in Swiss albino mice by weekly intra peritoneal (i.p.) inoculation of 10⁶ cells/mouse after every ten days. DAL cells 9 days old were used for the screening of the anticancer activity.

Acute oral toxicity study

Acute oral toxicity was performed by following OECD guideline - 420 fixed dose procedure for ethanol whole plant of *Polygala chinensis* and it was found that dose increasing up to 2000 mg/Kg body weight, shown no toxicity or mortality in experimental mice. The LD 50 of ethanol extracts of whole plant of *Polygala chinensis* as per OECD guidelines - 420 is greater than 2000 mg/Kg^{7,8}.

Antitumor activity

Healthy Swiss albino mice were divided in to six groups of six animals (n = 6) each. The test samples were dissolved in isotonic saline (0.9% NaCl W/V) and used directly in the assay. DAL cells were collected from the donor mouse and were suspended in sterile isotonic saline. The viable DAL cells were counted (Trypan blue indicator) under the microscope and were adjusted at 1×10^6 cells/ mL. 0.1 mL of DAL cells per 10 g body weight of the animals were injected (i.p.) to each mouse of each group except normal saline group (**Group I**). This was taken as Day 0. Group I served as a normal saline control (1 mL/Kg, p.o) and **Group II** served as DAL bearing control. On day 1, the ethanol extracts of *Polygala chinensis* at a dose of 100 and 200 mg/Kg each of the **Group III, IV** were administrated orally and continued for 14 consecutive days respectively. **Group V** served as tumor induced animal administrated with vincristine (80 mg/Kg body weight) for 14 consecutive days. On day 15, half of the animals (n = 3) in each case were sacrificed and the remaining animals were kept to observe the life span study of the tumor hosts. The effect of ethanol extract of *Polygala chinensis* on tumor growth and host's survival time were monitored by studying parameters like tumor volume, tumor cell count, viable tumor cell count, nonviable tumor cell count, mean survival time and increase in life span^{9,10}.

Tumor growth response

The effect of ethanol extracts of *Polygala chinensis* on tumor growth and hosts survival time were examined by studying the following parameters such as tumor volume, tumor cell count, viable tumor cell count, non viable tumor cell count, median survival time and increase in life span.

Determination of tumor volume

The mice were dissected and the ascitic fluid was collected from the peritoneal cavity. The volume was measured by taking it in a graduated centrifuge tube. Packed cell volume was determined by centrifuging the ascitic fluid at 1000 rpm for 5 min.

Determination of tumor cell count

The ascitic fluid was taken in a WBC pipette and diluted 100 times. Then a drop of the diluted cell suspension as placed on the Neubauer counting chamber and the number of cells in the 64 small squares was counted.

Estimation of viable and non-viable tumor cell count (Tryphan blue dye assay)

The cells were then stained with tryphan blue (0.4% normal saline) dye. The cells that did not take up the dye were viable and those that took the stain were non viable. These viable and non viable cells were counted.

Percentage increase of life span (% ILS)

Animals were inoculated (1×10^6 cells/mL) 0.1 mL of DAL cells per 10 g body weight of the animals was injected (i.p.) on day zero (day 0). A day of incubation was allowed for multiplication of the cells. Fourteen doses of the Test samples (100 mg/Kg and 200 mg/Kg, 0.1 mL/10 g body weight) and

control group was treated with same volume of Saline (0.9% sodium chloride solution) and compared with Vincristine (80 mg/Kg body weight) were injected i.p from the first day up to the 9th day with 24 h intervals. The effect of ethanol extracts of whole plant of *Polygala chinensis* tumor growth was monitored by recording the mortality, daily for a period of 9 days and percentage increase in life span (% ILS) was calculated from the following equation.

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

Body weight

Body weights of the experimental mice were recorded both in the treated and control group at the beginning of the experiment (zero day) and sequentially on every 5th day during the treatment period.

Hematological studies

At the end of the experimental period, all mice were sacrificed by cervical dislocation. Blood was collected from freely flowing tail vein and used for the estimation of Hemoglobin content (Hb), Red blood cell count (RBC) and White blood cell count (WBC). WBC differential toxicity¹¹.

Statistical analysis

The data were analyzed using student's t- test statistical methods. For the statistical tests, *p* values of less than 0.01 and 0.05 were taken as significant.

RESULTS AND DISCUSSION

The acute toxicity study, ethanol extracts of *Polygala chinensis* whole plant did not show any toxic effect up to the dose of 2000 mg/Kg body weight, according to 100 mg/Kg and 200 mg/Kg body weight were taken as low and high dose of whole plant *Polygala chinensis* for the experiment. The present investigation indicates that ethanol extracts of whole plant of *Polygala chinensis* showed significant antitumor activity in DAL bearing mice.

The administration of ethanol extract of whole plant of *Polygala chinensis* to DAL bearing mice showed reduction in body weight, spleen, thymus, liver, kidney and lungs (Table 1). In the case of tumor growth response study, treatment with ethanol extract of whole plant of *Polygala chinensis* showed significant (*p* < 0.01) reduction in tumor volume (Table 2). Table 3 depicts the effect of ethanol extract of whole plant of *Polygala chinensis* on life span, viable cell count and non viable cell count. It revealed that there was an increase in mean survival time. Administration of ethanol extract of *Polygala chinensis* appreciably decreases the viable cell count compared to DAL bearing mice. Non viable cell count was significantly higher with an increase in dosage of extracts. Table 4 showed that hematological parameters of tumor bearing mice on day 15 were found to be significantly different as compared to the extracts treated groups. In tumor bearing mice, it was found that there was an increase in WBC count, and a decrease in Hb content and RBC count. In differential count of WBC, the presence of neutrophils and monocytes increased while the lymphocyte count decreased in the DAL control group. Treatment with *Polygala chinensis* whole plant at the dose 100 mg/Kg and 200 mg/Kg significantly (*p* < 0.05 and *p* < 0.01, respectively) increased the Hb and RBC count to normal levels. The total WBC count was found to be increased significantly in the DAL control group when compared to the normal group. Administration of *Polygala chinensis* whole plant extracts (100 mg/Kg and 200 mg/Kg) in DAL bearing mice significantly (*p* < 0.05 and *p* < 0.01) reduced the WBC count as compared with DAL control.

Table 1: Effect of ethanol extract of *Polygala chinensis* on relative organ weights of tumor induced and drug treated mice

Treatment	Relative Organ Weight (g/100 g body wt.)					
	Body weight (g)	Spleen	Thymus	Liver	Kidney	Lungs
Group I	20.14 ± 1.75	0.39 ± 0.031	0.10 ± 0.037	2.78 ± 0.71	0.93 ± 0.20	0.41 ± 0.021
Group II	34.54 ± 1.22 ^a	0.48 ± 0.019	0.19 ± 0.051 ^a	3.41 ± 0.58 ^a	1.86 ± 0.11 ^a	0.68 ± 0.032 ^a
Group III	24.36 ± 0.63*	0.33 ± 0.013	0.15 ± 0.031	2.94 ± 0.26	1.03 ± 0.026	0.54 ± 0.021
Group IV	19.67 ± 0.74**	0.31 ± 0.043	0.11 ± 0.032*	2.77 ± 0.76*	0.85 ± 0.25*	0.42 ± 0.011
Group V	20.98 ± 1.86**	0.36 ± 0.023	0.14 ± 0.015	2.81 ± 0.64	1.09 ± 0.029	0.45 ± 0.034

Each value is SEM ± 6 individual observations: * $P < 0.05$; ** $P < 0.01$; Compared to DAL control vs drug treated groups a : $P < 0.05$; Compared to DAL control vs normal control

Table 2: Antitumor activity of ethanol extract of *Polygala chinensis* on solid tumor volume in tumor induced mice

Treatment	Solid tumor volume			
	15 th day	20 th day	25 th day	30 th day
Group II	3.65 ± 0.16	3.98 ± 0.27	4.36 ± 0.53	4.98 ± 0.53
Group III	3.14 ± 0.11*	3.16 ± 0.21	2.86 ± 0.16	2.52 ± 0.13
Group IV	2.87 ± 0.14**	2.93 ± 0.42**	2.48 ± 0.17**	2.23 ± 0.14**
Group V	3.18 ± 0.21 ^{NS}	3.04 ± 0.14**	2.63 ± 0.53**	2.31 ± 0.49**

Each Value is SEM of 6 animals Significance between tumor induced control vs drug treated group

* $P < 0.05$; ** $P < 0.01$, NS- Not Significant

Table 3: Antitumor activity of ethanol extract of *Polygala chinensis* on the survival time, life span, tumor volume and viable and non-viable cell count in tumor induced mice

Treatment	Mean survival time (Days)	Increase of life span (%)	Packed cell volume	Viable cell count X 10 ⁶ cells/mL	Non-viable tumor cells count X 10 ⁶ cells/mL
Group II	17.21 ± 0.56	-	3.20 ± 0.031	14.68 ± 2.01	0.83 ± 0.031
Group III	26.36 ± 0.18*	53.17	9.21 ± 0.016*	6.56 ± 0.18*	2.31 ± 0.21*
Group IV	32.74 ± 0.23**	90.23	0.91 ± 0.056**	5.81 ± 0.39**	3.12 ± 0.067*
Group V	30.68 ± 0.45*	78.26	0.98 ± 0.074**	6.54 ± 0.66**	2.89 ± 0.038*

Each Value is SEM of 6 animals Significance between tumor induced control vs drug treated group

* $P < 0.05$; ** $P < 0.01$

Table 4: Anticancer activity of ethanol extract of *Polygala chinensis* on hematological parameters in DAL tumor bearing mice

Parameter	Hb (gm%)	RBC (million/mm ³)	W WBC (10 ³ cells/ mm ³)	Differential count		
				Lymphocytes	Neutrophils	Eusinophill
Group I	14.34 ± 0.46	3.96 ± 0.24	9.67 ± 0.68	52.18 ± 1.44	43.81 ± 0.97	4.35 ± 0.74
Group II	7.94 ± 0.76**	2.97 ± 0.17*	16.56 ± 0.95	30.29 ± 1.34	62.32 ± 1.51	7.36 ± 0.87
Group III	11.52 ± 0.56*	3.86 ± 0.14*	10.55 ± 0.58*	45.30 ± 1.18	49.31 ± 1.21	5.38 ± 0.34
Group IV	10.98 ± 0.93*	3.64 ± 0.65	12.66 ± 0.89	41.56 ± 1.77	53.11 ± 1.43	6.02 ± 0.64
Group V	13.04 ± 0.55**	4.13 ± 0.65**	8.91 ± 0.33*	50.48 ± 1.32	41.23 ± 1.37	7.84 ± 0.47

Each Value is SEM of 6 animals Significance between tumour induced control vs drug treated group

* $P < 0.05$; ** $P < 0.01$

The present study was carried out to investigate the antitumor potential of whole plant of *Polygala chinensis* against DAL bearing mice. The ethanol extract treated animals at the doses of 100 and 200 mg/Kg significantly inhibited the tumor volume, packed cell volume, tumor (viable) cell count and brought back the hematological parameters to more or less normal levels.

In DAL tumor bearing mice, a regular rapid increase in ascitic tumor volume was observed. Ascitic fluid is the direct nutritional source for tumor cells and rapid increase in ascitic fluid with tumor growth would be a means to meet the nutritional requirement of tumor cells¹². Treatment with ethanol extract of *Polygala chinensis* inhibited the tumor volume, viable tumor cell count and increased the life span of the tumor bearing mice. The reliable criteria for judging the value of any anticancer drug are the prolongation of the life span of animals¹³. It may be concluded that ethanol extract of *Polygala chinensis* by decreasing the nutritional fluid volume and arresting the tumor growth increases the life span of DAL bearing mice. Thus, ethanol extract of *Polygala chinensis* has antitumor activity against DAL bearing mice.

Usually, in cancer chemotherapy the major problems that are being encountered are of myelosuppression and anemia¹⁴⁻¹⁶. The anemia encountered in tumor bearing mice its mainly due to reduction in RBC or hemoglobin percentage, and this may occur either due to iron deficiency or due to hemolytic or myelopathic conditions^{16,17}. Treatment with ethanol extract of *Polygala chinensis* whole plant brought back the hemoglobin (Hb) content, RBC and WBC count more or less to normal levels. This clearly indicates that *Polygala chinensis* whole plant possess protective action on the hemopoietic system. 1,5-Anhydro-d-mannitol, 9,12-Octadecadienoic acid (Z,Z)-, Phytol, Oleic acid, Squalene, Ethyl iso-albeholate and 6,7-Epoxypregn-4-ene-9,11,18-triol-3,20-dione, 11,18-diacetate were reported in the ethanol extract of *Polygala chinensis* whole plant by GC-MS analysis. Three compounds may have the role in anticancer activity¹⁸. Further, the isolation of the compounds responsible for anticancer activity has to be taken up, which may result in a modern drug from this plant.

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