

# Antitumor activity of *Eugenia floccosa* Bedd and *Eugenia singampattiana* Bedd leaves against Dalton ascites lymphoma in swiss albino mice

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**Abstract:** The aim of the present study is to evaluate the antitumor effect of *Eugenia floccosa* and *Eugenia singampattiana* (Myrtaceae) leaves against DLA bearing Swiss Albino Mice. The effect of ethanol extracts of *Eugenia floccosa* and *Eugenia singampattiana* leaves on tumor growth and host's survival time was studied by the following parameters: tumor volume, viable and non viable cell count and life span of the host. Ethanol extracts of *Eugenia floccosa* and *Eugenia singampattiana* and combined extracts of *Eugenia floccosa* and *Eugenia singampattiana* were administered at a 100mg/Kg body weight respectively once a day for 14 days, after 24h of tumor inoculation. Decrease in tumor volume and viable count were observed. Treatment with the above said extracts increase the mean survival time. Hematological studies reveal that the Hb content was decreased in DAL treated mice, whereas, restoration to near normal levels was observed in extracts treated animals. The results suggest that the ethanol extracts of *Eugenia floccosa* and *Eugenia singampattiana* leaves exhibits significant antitumor effects in DAL bearing mice.

**Keywords:** Antitumor, DAL, Haematological studies.

## Introduction

Cancer is one of the most life-threatening diseases and serious public health problems in both developed and developing countries. It is a group of diseases characterized by the disregulate proliferation of abnormal cells that invade and disrupt surrounding tissues<sup>1</sup>. Due to the toxic and adverse effects of synthetic drugs as well as conventional treatments are being failed to fulfill their objective for these consequence herbal medicine has made a comeback to improve the fulfillment of our present and future health needs<sup>2</sup>.

Inspite of the recent domination of the synthetic chemistry as a method to discover and produce drugs, the potential of bioactive plants or their extracts to provide new and novel products for disease treatment and prevention is still enormous<sup>3</sup>. The antitumor area has the greatest impact of plant derived drugs, where drugs like vinblastine, vincristine, taxol and camptothecin have improved the chemotherapy of some cancers<sup>4</sup>. Plants have an almost unlimited capacity to produce substances that attract researchers in the quest for new and novel chemotherapeutics<sup>5</sup>. The present study was conducted to evaluate the antitumor activity of ethanolic extracts of *Eugenia floccosa* Bedd and *Eugenia singampattiana* Bedd

leaves against Dalton ascites lymphoma (DAL) in swiss albino mice. So far no reports are available in antitumor activity of these two plants against DAL.

## **Methodology**

### **Anticancer activity**

#### **Animals**

Male adult Swiss Albino mice (20-25 gm) were procured from Animal Experimental Laboratory of Raja Muthaiya Medical College, Annamalai University Chidambaram Tamil Nadu and used throughout the study. They were housed in microcolon boxes in a controlled environment (temperature  $25 \pm 2$  °C) and 12 hr dark/light cycle) with standard laboratory diet (SaiDurga feeds and Foods, Bangalore) and water *ad libitum*. The study was conducted after obtaining institutional animal ethical committee's clearance as per the standard practice, the mice were 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) cells were obtained under the courtesy of Department of Biochemistry Adiar Cancer Institute Chennai, India. The freshly drawn ascitic fluid was diluted in phosphate buffer solution pH (6.8) and aliquote of ( $1 \times 10^6$  cells 0.25 ml) of the diluted solution were injected intraperitoneal inoculation to mice belonging to age group of 5 to 6 weeks and weight (20 to 25 gms)

#### **AntitumorActivity**

After acclimatization, mature male Swiss albino mice were divided into four groups (n=10). All the groups except group I, were injected with DAL cells ( $1 \times 10^6$  cells/mouse.i.p.). This was taken as day 0. Group I served as normal saline control (1 ml/kg, p.o.) and group II served as DAL bearing control. On day 1, the ethanol extracts of *Eugenia floccosa* and *Eugenia singampattiana* at a dose of 100 mg/kg each and combined extract of 100+100 mg/kg body weight to the Group III, IV and V were administered orally and continued for 14 consecutive days respectively. Group VI served as tumor induced animal administered with 5-fluorouracil (20mg/kg body weight) for 14 consecutive days. On day 15, five mice of each group were sacrificed 24 hours after the last dose and the rest were kept with food and water *ad libitum* to check the increase in the life span of the tumor hosts. The effect of ethanol extract of *Eugenia floccosa* and *Eugenia singampattiana* on tumor growth and host's survival

time were monitored by studying parameters like tumor volume, tumor cell count, viable tumor cell count, non viable tumor cell count, mean 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 and packed cell volume determined by centrifuging at 1000 g for five minutes.

#### **Determination of Tumor Cell Count**

The ascitic fluid was taken in a haematocrit (micro) tube and diluted 1000 times. Then a drop of the diluted cell suspension was placed on the Neubauer counting chamber and the cells in 64 small squares were counted.

#### **Estimation of Viable and Non-viable Tumor Cell Count**

The cells were then stained with 0.4% trypan blue in physiological saline. The dye was counted as viable and nonviable cell count. The cells that did not take up the dye were viable and those that took the stains were non viable. These viable and non viable cells were counted.

#### **Cell count =**

No. of cells X Dilution

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Area X Thickness of liquid film

#### **Percentage of increase life span (% ILS)**

The effect of ethanol extracts of *Eugenia floccosa* and *Eugenia singampattiana* tumor growth was monitored by recording the mortality daily and percentage increase in the life span (% ILS) was calculated.

#### **% ILS=**

Mean survival of treated group

----- X 1 X 100

Mean survival of control group

#### **Mean survival =**

Day of 1<sup>st</sup> death + Day of last death

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### Haematological Studies

Red blood cell count (RBC), haemoglobin content and white blood cell (WBC) counts were measured from freely flowing tail vein blood. WBC differential count was carried out from Leishman stained blood smears. Protein concentration was estimated by Lowry's method<sup>6</sup>. One millilitre of peritoneal fluid was withdrawn and centrifuged at 3000 rpm for 30 min according to the method described by Dacie and Lewis<sup>7</sup>.

### Results

#### Anticancer activity

Antitumor activity of ethanol extracts of leaves of *Eugenia floccosa*, *Eugenia singampattiana* and combined extracts of *Eugenia floccosa* and *Eugenia singampattiana* against DAL tumor bearing mice was assessed by the parameters such as tumor volume, viable and non viable cell count, mean survival time and % increase of life span. The results are shown in table 1. The tumor volume and viable cell count were found to be significantly increased and non viable cell count was significantly low in DAL control animals when compared with normal control animals.

Administration of ethanol extracts of *E. floccosa* (100mg/kg), *E. singampattiana* (100mg/kg) and combined extract (100+100mg/kg), significantly ( $p < 0.05$ ) decrease the tumor volume and viable cell count. Non-viable cell count was significantly ( $p < 0.05$ ) higher in all the three extracts treated animals when compared with DAL control animals. The mean survival time was increased to  $28.45 \pm 1.63$  (% ILS=44.30),  $24.60 \pm 1.33$  (% ILS=40.15),  $35.60 \pm 1.59$  (% ILS=63.21) and  $34.10 \pm 1.34$  (% ILS=59.33) on administration of ethanol extracts of *E. floccosa*, *E. singampattiana*, combined extracts and 5-fluorouracil respectively.

Haematological parameters (Table 2) of tumor bearing mice (Group II) on day 14 were found to be significantly altered from normal group (Group I). The total WBC count was found to be increased with a reduction of Hb content of RBC. The total number of RBC showed a modest change. In differential count of WBC, the per cent of neutrophils increased while the lymphocyte count decreased. At the same time, administration of combined extracts of *E. floccosa* and *E. singampattiana* (Group V) treatment also recovered these altered depleted parameters towards near normal.

**Table 1: Effects of ethanol extract of *Eugenia floccosa* and *Eugenia singampattiana* leaves on the survival term, life span, tumor volume and viable and non-viable cell count for DAL Tumor induced mice**

Parameter	Survival time (Days)	Increase of life span(%)	Tumor volume(ml)	Viable cell count $1 \times 10^6$ cells/ml	Non-Viable cell count $1 \times 10^6$ cells/ml
Group I	—	—	—	—	—
Group II	$18.50 \pm 1.31$	—	$4.26 \pm 0.12$	$10.36 \pm 0.11$	$1.59 \pm 0.32$
Group III	$28.45 \pm 1.63^*$	44.30	$1.65 \pm 0.16^*$	$03.31 \pm 0.31^*$	$1.86 \pm 0.16^*$
Group IV	$24.60 \pm 1.33^*$	40.15	$2.13 \pm 0.12^*$	$4.11 \pm 0.14^*$	$2.31 \pm 0.17^*$
Group V	$35.6 \pm 1.59^*$	63.21	$1.13 \pm 0.09^*$	$2.36 \pm 0.30^*$	$2.69 \pm 0.13^*$
Group VI	$34.1 \pm 1.34^*$	59.33	$1.25 \pm 0.05^*$	$2.05 \pm 0.19^*$	$2.94 \pm 0.21^*$

Statistical significance (P) calculated by oneway ANOVA followed by Dunnett's test.

$p < 0.05$  calculated by comparing treated groups with DAC control groups.

**Table 2: Effect of ethanol extract of *Eugenia floccosa* and *Eugenia singampattiana* leaves on haematological parameters in DAL Tumor bearing mice**

Parameter	Hb (gm%)	RBC (million/mm <sup>3</sup> )	WBC (10 <sup>3</sup> cells/mm <sup>3</sup> )	Proteins (gm%)	Differential count		
					Lymphocytes (%)	Neutrophils (%)	Monocytes (%)
Group I	15.6 ± 0.3	6.9 ± 0.4	8.4 ± 0.3	7.8 ± 0.4	73.1 ± 1.9	25.3 ± 1.2	1.1 ± 0.3
Group II	6.5 ± 0.6**	2.9 ± 0.1	16.5 ± 1.1**	14.3 ± 1.2**	29.5 ± 1.3 **	64.6 ± 1.4 **	7.3 ± 0.2 *
Group III	11.4 ± 0.4**	5.8 ± 0.2*	9.3 ± 0.8**	9.1 ± 0.6**	61.4 ± 1.8 **	37.2 ± 1.1**	1.9 ± 0.1NS
Group IV	10.9 ± 0.3**	5.1 ± 0.1*	10.1 ± 0.3*b	9.9 ± 0.3*	64.2 ± 1.5**	33.3 ± 1.6**	2.1 ± 0.2NS
Group V	14.5 ± 0.2**	6.5 ± 0.3**	8.1 ± 0.2**	8.1 ± 0.4**	69.5 ± 1.6**	27.5 ± 0.8**	3.5 ± 0.1
Group VI	13.3 ± 0.5**	6.2 ± 0.1*	8.6 ± 0.1**	8.3 ± 0.2**	70.5 ± 1.2**	24.6 ± 0.7**	4.9 ± 0.2

Statically significance (P) calculated by one way ANOVA followed by Dunnett's test. p < 0.01 calculated by comparing treated groups with DAC control groups. \*\* p < 0.01

## Discussion

### Anticancer activity

The present investigation was carried out to evaluate the antitumor activity of ethanol extracts of leaves of *Eugenia floccosa*, *Eugenia singampattiana* and combined extracts in DAL tumor bearing mice. The ethanol extracts of above said plants treated animals at the dose of 100 mg/kg significantly inhibited the tumor volume and tumor (viable) cell count and brought back the haematological parameter to more or less normal levels.

In DAL tumor bearing mice, a regular rapid increase in ascetic tumor volume was observed. Ascetic fluid is the direct nutritional source for tumor cells and a rapid increase in ascetic fluid with tumor growth would be a means to meet the nutritional requirement of tumor cells<sup>8</sup>. Treatment with ethanol extracts of *E. floccosa* and *E. singampattiana* leaves 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 animals<sup>9</sup>. It may be concluded that ethanol extracts of *E. floccosa* and *E. singampattiana* by decreasing the nutritional fluid volume and arresting the tumor growth and increase the life span of DAL bearing mice. Thus, ethanol extracts of *E. floccosa* and *E. singampattiana* has antitumor activity against DAL bearing mice.

In cancer chemotherapy the major problems that are being encountered are of myelosuppression and anaemia<sup>10, 11</sup>. The anaemia encountered in tumor bearing mice is mainly due to reduction in RBC or haemoglobin percentage and this may occur either due to iron deficiency order to haemolytic or myelopathic conditions<sup>12</sup>. Treatment with ethanol extracts of *E. floccosa*, *E. singampattiana* and combined extracts of brought back the haemoglobin (Hb) content, RBC and

WBC count more or less to normal levels. This clearly indicates that ethanol extracts of *E. floccosa* and *E. singampattiana* posses protective action on the haemopoietic system.

The results of the present study demonstrates that the ethanol extracts of *E. floccosa* and *E. singampattiana* increased the life span of DAL tumor bearing mice, reduce tumor volume and improve the haematological parameters. The association between flavonoids and reduced cancer risk has been reported in previous studies that showed a decrease in cancer risk with consumption of vegetables and fruits rich with flavonoids<sup>13,14</sup>. The results of this study are accordance with this finding since the phytochemical screening showed the presence of flavonoids in ethanol extracts of *E. floccosa* and *E. singampattiana*. While the presence of alkaloids with flavonoids in *E. floccosa* and *E. singampattiana* extracts may explain its superior activity compared with other plants studied<sup>15</sup>. The anticancer activity of total flavonoids and alkaloids isolated from different plants were reported earlier<sup>16, 14</sup>. Plants derived compounds have played an important role in the development of several clinical useful anticancer agents<sup>17</sup>. Since the phytochemical screening, *E. floccosa* and *E. singampattiana* showed the presence of alkaloids, flavonoids, terpenoids, steroids, saponins, glycosides and phenols which could make the plants useful for treating anticancer drug. Further, the isolation of the compounds responsible for the activity has to be taken up which may result in a modern drug from these plants.

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