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Research Article

**ANTICANCER ACTIVITY OF METHANOL EXTRACT OF
SESUVIUM PORTULACASTRUM L. WHOLE PLANT AGAINST
EHRlich ASCITES CARCINOMA (EAC)**

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*Head of the Department of Botany (Rtd) St. Mary's College (Autonomous) Thoothukudi,
Tamilnadu, India.**Abstract:**

Aim: To study aims to evaluate the antitumor activity of methanol extract of *Sesuvium portulacastrum* whole plant (Family: Aizoaceae) on EAC model in Swiss Albino mice. **Methods:** Evaluation of the antitumor effect of methanol extract of whole plant of *Sesuvium portulacastrum* 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. **Results:** The results showed decrease in tumor volume and cell viability. Hematological studies revealed that, the Hb count decreased in EAC treated mice, whereas, it was induced by the drug treated animals and showed an increase in Hb near to normal levels. **Conclusion:** The results suggested that, the extracts of whole plant of *Sesuvium portulacastrum* exhibited significant antitumor activity on EAC bearing mice.

Keywords: *Sesuvium portulacastrum*, antitumor, lifespan, WBC.**Corresponding author:****Dr. D.Sheela,***Head of the Department of Botany (Rtd),
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INTRODUCTION:

Cancer is considered one of the most common causes of mortality worldwide. Progress made in cancer therapy has not been sufficient to a significantly lower annual death rate from most tumor types, and there is an urgent need for new strategies in cancer control [1]. For centuries, people have been using plants for their therapeutic values. Today 85000 plants have been documented for therapeutic use globally [2]. The World Health Organization (WHO) estimates that almost 75% of World's population has therapeutic experience with herbal drugs. Cancer is one of the most dangerous diseases in humans and presently there is a considerable scientific discovery of new anticancer agents from natural products [3]. The potential of using the natural products as anticancer drugs was recognized in 1950's by U.S. Natural Cancer Institute (NCI) since 1950 major contributions have taken for the discovery of naturally occurring anticancer drugs [4].

Sesuvium portulacastrum belongs to the Aizoaceae family. It is also called as "Sea purslane". It is a perennial herb found on the sea coasts. It grows on the ocean side of the dunes down to the high tide mark. It is commonly called "Orputu and Vankaravacci". This plant is used in traditional medicine as a remedy for fever, kidney disorders and scurvy [5] by the indigenous people in Africa, Latin America and in Asian countries such as India, China, Pakistan and Japan. Medicinally and economically, *Sesuvium* containing secondary metabolites has shown a great potential as a substitute for some synthetic raw materials in the food, perfumery, cosmetic and pharmaceutical industries [6,7]. *S.portulacastrum* whole plant may also have brought about hypoglycemic action through stimulation of surviving β -cells islets of Langerhans to release more insulin. This was clearly evidenced by the increased level plasma insulin in diabetic rats treated with *S.portulacastrum* [8]. Methanol extract of *S.portulacastrum* at the doses of 150kg/mg and the altered glutathione peroxidase, glutathione reductase, superoxide dismutase, catalase restored and decreased glutathione levels towards the normal levels in a dose dependent manner in rats using Silymarin as standard drug (9). Taking into consideration of the medicinal importance of *S.portulacastrum*, the methanol extract of the whole plant *S.portulacastrum* were analyzed for their anticancer activity against Ehrlich ascites carcinoma (EAC) tumor model.

MATERIALS AND METHODS:

Collection

The well grown whole plant of *Sesuvium portulacastrum* (L.) L was collected from coastal regions of Thoothukudi, District, Tamil Nadu.

With the help of local flora, voucher specimens were identified and preserved in the Research Department of Botany, St. Mary's College, Turicorin, Tamil Nadu for further references.

Preparation of plant extract for anticancer activity

The whole plant of *Sesuvium portulacastrum* were cut into small pieces, washed, dried at room temperature and the dried leaves were powdered in a Wiley mill. Hundred grams of powdered leaves were separately packed in a Soxhlet apparatus and extracted with methanol. The methanol extracts were concentrated in a rotary evaporator. The concentrated methanol extracts of whole plant were used for preliminary phytochemical screening and anticancer activity.

Animals

Healthy male adult Swiss Albino mice (20-25gm) were used for the study. The animals were housed in microloan boxes in a controlled environment (temperature $25\pm 20^{\circ}\text{C}$) and 12 hr dark/eight cycle) with standard laboratory diet (Sai Durga feeds and foods, Bangalore) and water *ad libitum*. 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

Ehrlich ascites carcinoma (EAC) cells were obtained under the courtesy of Department of Biochemistry, Adaiyar Cancer Institute, Chennai, Tamil Nadu, India. The EAC cells were maintained *in vivo* in Swiss albino mice by weekly intra peritoneal (i. p) inoculation of 10^6 cells / mouse after every ten days. EAC 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 - 20 fixed dose procedure for methanol extract of whole plant of *Sesuvium portulacastrum* and it was found that, dose increasing up to 2000 mg / kg body weight, shown no toxicity or mortality in experimental mice. The LD50 of methanol extracts of whole plant of *Sesuvium portulacastrum* as per OECD guidelines-420 is greater than 2000 mg/kg [10,11].

Antitumor activity

Healthy Swiss albino mice were divided into 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. EAC cells were collected from the donor mouse and were suspended in sterile isotonic saline. The viable EAC cells were counted (Trypan blue indicator) under the microscope and were

adjusted at 1×10^6 cells/ ml. 0.1 ml of EAC cells per 10g 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 (1mL/kg, p.o) and group II served as EAC bearing control. On day 1, the methanol extracts of *S.portulacastrum* at a dose of 150 and 300mg/kg each of the Group III, IV were administered orally and continued for 14 consecutive days respectively. Group V served as tumor induced animal administered with vincristine (80mg/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 methanol extract of *S.portulacastrum* 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 [12,13].

Tumor growth response

The effect of methanol extract of *S.portulacastrum* 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 5min.

Determination of Tumor cell count

The ascitic fluid was taken in a haematocrit (micro) tube and diluted 100times. 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 (Trypan blue dye assay):

The cells were then stained with trypan 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) 0.1ml of EAC cells per 10g 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 (150 mg/kg and 300 mg/kg, 0.1 ml/10g body weight) and control group was treated with same volume of Saline (0.9% sodium chloride solution) and compared with vincristine (80mg / kg body weight) were injected i.p from the first day up to the 9th day with 24 h intervals. The effect of methanol extracts of whole plant of *S.portulacastrum* 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.

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

C

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 Haemoglobin content (Hb), Red blood cell count (RBC) and White blood cell count (WBC). WBC differential count was carried out from Leishman stained blood smears [14].

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, methanol extract of *S.portulacastrum* whole plant did not show any toxic effect upto the dose of 2000mg/kg body weight, according to 150mg/kg and 300mg/kg were taken as low and high dose of whole plant of *S.portulacastrum* for the experiment. The present investigation indicates that methanol extract of whole plant of *S.portulacastrum* showed significant antitumor activity in EAC bearing mice.

Table 1: Effect of SPW extracts on relative organ weights of tumour induced (EAC) and drug treated mice.

Treatment/dose	Body weight(g)	Relative organ weight (g/100g b wt.)				
		Spleen	Thymus	Liver	Kidney	Lungs
NormalControl (Saline) (GroupI)	19.68±1.45	0.42±0.012	0.21±0.019	2.98±0.422	0.91±0.042	0.58±0.04
Tumor induced control (Saline) (Group11)	37.14±1.16***	0.89±0.042***	0.32±0.08*	3.49±0.417***	1.81±0.07*	0.81±0.05*
SPWextract(150mg/kg) +(Group111) EAC	32.58±1.71**	0.74±0.012**	0.33±0.07*	3.21±0.611	0.92±0.04 ^a	0.67±0.01 ^{ns}
SPWextract300mg/kg+(Group1V) EAC	36.33±1.51***	0.65±0.091*	0.29±0.76 ^{ns}	2.31±0.410 ^a	0.78±0.02 ^{aa}	0.42±0.01 ^a
Vincristine80mg/kg+(Group V) EAC	25.64±1.31 ^{ns}	0.44±0.021 ^{aa}	0.22±0.62 ^a	2.04±0.982 ^a	0.99±0.210 ^a	0.56±0.08 ^a

Each Value is SEM of 6 animals. Significance between normal control, tumor induced control vs drug treated group * $p < 0.05$; ** $p < 0.01$, ns- not significant. ^a $p < 0.05$; ^{aa} $p < 0.01$ - Significance between tumor induced control vs drug treated group. Ehrlich ascites carcinoma (EAC). SPW- Sesuvium portulacastrum whole plant.

Table-1 shows administration of methanol extract of whole plant of *S.portulacastrum* to EAC bearing mice showed reduction in bodyweight, spleen, thymus, liver,

kidney and lungs. The effects of methanol extract of *S.portulacastrum* whole plant at the doses of 150 and 300mg/kg on solid tumor volume is shown in Table-2.

Table 2: Antitumor activity of SPW extracts on tumor volume intumor (EAC) induced mice.

Treatment	Solid Tumor Volume			
	15th day	20th day	25th day	30th day
NormalControl (Saline) (GroupI)	-	-	-	-
Tumor induced control (Saline) (Group11)	5.08±0.024	6.93±0.22	8.44±0.42	10.36±0.91
SPWextract(150mg/kg)+(Group111) EAC	6.34±0.053	5.83±0.081	4.65±0.81**	3.24±0.18***
SPWextract300mg/kg+(Group1V) EAC	5.93±0.092	4.64±0.63*	3.21±0.43***	2.39±0.26***
Vincristine80mg/kg+(Group V) EAC	4.13±0.110 ^{ns}	3.04±0.710***	2.64±0.87***	2.02±0.51***

Each Value is SEM of 6 animals. Significance between tumor induced control vs drug treated group * $p < 0.05$; ** $p < 0.01$, Ehrlich ascites carcinoma (EAC). NS- not significant. SPW- Sesuvium portulacastrum whole plant.

Table 3: Antitumor activity of S.portulacastrum whole extract 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 tumorcells countX10 ⁶ cell/ ml
NormalControl (Saline) (GroupI)	-	-	-	-	-
Tumor induced control (Saline) (Group11)	18.61±0.41	-	2.86±0.045	15.81±0.33	0.98±0.11
SPWextract(150mg/kg) +(Group111) EAC	21.43±0.12	31.27	2.71±0.031	12.81±0.11	0.91±0.45
SPWextract300mg/kg+(Group1V) EAC	28.61±0.43***	42.98	1.70±0.091*	6.82±0.41**	1.19±0.81
Vincristine80mg/kg+(Group V) EAC	27.26±0.14***	51.85	1.22±0.61*	5.61±0.71**	1.67±0.91*

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. SPW- Sesuvium portulacastrum whole plant.

Treatment with methanol extract of whole plant of *S.portulacastrum* and vincristine at the dose of 150 and 300mg/kg significantly ($P<0.01$) reduces the solid tumor volume in a dose dependent manner as compared to that of the EAC control group. The effects of methanol extract of *S.portulacastrum* whole plant at the doses of 150 and 300mg/kg on Survival time (days), Life span (%), Packed cell volume, tumor cell count(viable and non viable cell) shown in Table-3.

In the EAC control group, the mean survival time was 18.61 ± 0.41 days, while it increased 21.43 ± 0.12 (150mg/kg) and 28.61 ± 0.43 (300mg/kg) days respectively, in the methanol extract of *S.portulacastrum* treated groups, whereas the standard drug vincristine (80mg/kg) treated group had a mean survival time of 27.26 ± 0.14 days. The percentage increase in survivals, it was found to be 31.27%, 42.98% and 51.85% respectively as compared to EAC control group. Treatment with methanol extract of *S.portulacastrum* whole plant at the doses of 300mg/kg significantly ($P<0.01$) reduced the packed cell volume and viable tumor cell count in a dose dependent manner as compared to that of the EAC control group. Furthermore, nonviable cell counts at different doses of methanol extract of *S.portulacastrum* were increased in a dose dependent manner. As shown in (Table-4) RBC, HB, lymphocytes were decreased and WBC count, Neutrophil, Eosinophil were significantly increased in the EAC control group compared to the normal control group.

Treatment with methanol extract of whole plant of *S.portulacastrum* at the dose of 150 and 300mg/kg significantly increases in the HB count and RBC significantly decreased the WBC count to about normal level. All these results suggest the anticancer nature of the extract. However, the standard vincristine at the

dose of 80mg/kg body weight produced better result in all these parameters. The alternative system of medicines like Ayurvedic, Siddha, Unani and other tribal folklore medicines have significantly contributed to the health care of the population of India. Today these systems are not only complementary but also competitive in the treatment of various diseases. Plants have served as a good source of antitumor agents. Several studies have been conducted on herbs under a multitude of ethanobotanical grounds. A large number of plants possessing anticancer properties have been documented [15-20]. The present investigation was carried out to evaluate the antitumor activity of methanol extract of *S.portulacastrum* in EAC tumor bearing mice. The methanol extract of *S.portulacastrum* treated animals at the doses of 150 and 300 mg/kg significantly decreased the tumor volume, packed cell volume, tumor (viable) cell count and brought back the hematological parameters to more or less normal levels. In EAC tumor bearing animals a regular rapid increase in ascitic tumor volume was observed. Ascitic fluid is the direct nutritional source for tumor cells and a rapid increase in ascitic fluid with tumor growth would be a means to meet the nutritional requirement of tumor cells [21]. Treatment with methanol extract of *S.portulacastrum* 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 lifespan of animals [22]. It may be concluded that methanol extract of *S.portulacastrum* by decreasing the nutritional fluid volume and arresting the tumor growth increases the life span of EAC bearing mice. Thus methanol extract of *S.portulacastrum* have antitumor activity against EAC bearing mice. Usually, in cancer chemotherapy the major problems that are being encountered are myelosuppression and anemia [23-24].

Table 4: Antitumor activity of SPW on haematological parameters in Tumor (EAC) bearing mice

Parameter	Hb (gm %)	RBC (million/mm ³)	WBC (10 ³ cells/ mm ³)	Differential count		
				Lymphocytes	Neutrophils	Eosinophils
NormalControl (Saline) (GroupI)	10.61±0.21	3.15±0.62	7.14±0.43	53.61±1.93	40.15±0.17	3.31±0.13
Tumor induced control (Saline) (Group11)	5.62±0.41**	2.61±0.22*	12.71±0.31**	50.68±0.32	46.41±0.82	3.52±0.53
SPWextract(150mg/kg)+ (Group111) EAC	7.41±0.31	2.74±0.12	10.21±0.41 _{ns}	43.11±0.61	52.11±0.31	4.74±0.22
SPW extract 300mg/kg+(Group1V) EAC	10.27±0.48	3.41±0.21	7.24±0.44 ^a	54.61±0.41	40.48±0.47	5.77±0.35
Vincristine 80mg/kg+(GroupV) EAC	11.40±0.71	4.23±0.61	8.12±0.71 ^a	52.32±0.61	40.61±0.21	6.71±0.14 ^a

Each Value is SEM of 6 animals. Significance between normal control, tumor induced control vs drug treated group * $p < 0.05$; ** $p < 0.01$; NS -Not significant, ^a $p < 0.05$; ^{aa} $p < 0.01$ - Significance between tumor induced control vs drug treated group. Ehrlich ascites carcinoma (EAC). SPW- Sesuvium portulacastrum whole plant.

The anemia encountered in tumor bearing mice is mainly due to reduction in RBC or Hb and this may occur either due to iron deficiency or due to hemolytic or myelopathic conditions [25]. In EAC control group, a differential count the presence of neutrophils increased, while the lymphocyte count decreased, the observed leucocytopenia indicates a common symptom of immunosuppression in many types of cancer [26-27] and one of the causes of neutrophilia is myeloid growth factors which are produced in malignant process as part of a paraneoplastic syndrome. In addition to this another factor granulocyte colony stimulating factor produced by the malignant cells has also been attributed to be the cause of neutrophilia because of its action on bone marrow granulocytic cells in cancer. After the repeated, methanol extract of *S.portulacastrum* able to reverse the changes in altered neutrophils and lymphocytes count [28-29]. Treatment with both doses of methanol extract of *S.portulacastrum* brought back the Hb content, RBC and WBC count more or less to normal levels significantly.

This clearly indicates that methanol extracts of *S.portulacastrum* possess protective action on the haemopoietic system. Plant derived compounds have played an important role in the development of several clinical useful anticancer agents [30]. Phytol, 9, 12, 15. Octadecatrienoic acid, 2, 3, dihydroxypropyl ester, (Z,Z,Z), Oleic acid, eicosyl ester, squalene, vitamin E were reported in the methanol extract of *S.portulacastrum* whole plant by GC-MS analysis. These compounds may play a role in anticancer activity [31].

CONCLUSION:

The present study concluded that the methanol extract has shown a remarkable anticancer activity against the experimental cells namely Ehrlich ascites carcinoma (EAC). This holds great promise for future research in human beings.

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