

Anticancer Potential of *Drosera peltata* J. E. Sm against Ehrlich's Ascites Carcinoma (EAC) Tumor in Mice

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Abstract: In the present study was carried out to evaluate the anticancer potential of *Drosera peltata* J.E.Sm against Ehrlich's Ascites Carcinoma (EAC) induced tumor in mice. The ethanol and aqueous extract of *Drosera peltata* J.E.Sm. was given orally to mice at the dose of 250, 500 mg/kg body weight for 14 days to EAC bearing mice (4 Groups n=10) and 20mg/kg of 5-Fluorouracil (1 group as standard). Phytoconstituents of both extracts were analyzed through HPTLC methods using plumbagin and quercetin as a standard marker. Treatment caused significant reduction in body weight, packed cell volume (PCV) and viable tumor cell count when compared to the mice of the EAC control group. Restoration of hematological parameters towards normal was also observed. The dose at 250,500mg/kg of ethanol extracts and 500mg/kg of aqueous extract showed significant ($p<0.001$) result when compared with 250mg/kg of aqueous extract dose. HPTLC study confirmed that presence of plumbagin and quercetin in ethanol and aqueous extract. The results concluded that the ethanol and aqueous extracts of *Drosera peltata*, exhibited significant anticancer activity in EAC bearing mice which might be the presence of phytoconstituent plumbagin and quercetin.

Key words: *Drosera peltata*, Ehrlich's Ascites Carcinoma, HPTLC, Plumbagin, anticancer.

INTRODUCTION

India is a rich source of medicinal plants, from which natural and derived products such as flavonoids, alkaloids, terpenes and have received considerable attention in recent years due to their diverse pharmacological properties including cytotoxicity and cancer chemoprotective effects.

Flavonoids are nearly ubiquitous in plants[1]. Many drugs are derived from natural products, such as Paclitaxel, a diterpenoid from *Taxus brevifolia*, Vincristine and Vinblastine from *Catharanthus roseus*, Camptothecin from *Camptotheca acuminata* amongst others for the treatment of cancer[2]. *Drosera* species are known as sundew plant belonging to family Droseraceae. It consists of approximately 170 species. Three species of *Drosera* are found in India viz., *D.burmannii* Vahl, *D. indica* L., and *D. peltata* J.E.Sm.. It contains 1, 4-naphthoquinones, plumbagin, ramantaceon and its glucosiderossoliside, flavonoids like quercetin and hyperoside. Plumbagin is 5-hydroxy-2-methyl-1,4-naphthoquinone, a yellow colored pigment found in Plumbaginaceae and Droseraceae. *Drosera* species are used as vital components in an Ayurvedic preparation called 'Swarnabhasma' (Golden ash) are used for the treatment of different diseases like bronchial asthma, rheumatoid arthritis, diabetes mellitus, nervous disorders[3]. *Drosera peltata* extract could be used in the treatment of oral infectious diseases like dental caries and periodontitis[4]. A vast

literature collection fails to produce a scientific evidence to prove the anticancer activity of *D. peltata* extracts. Hence this study was planned to evaluate the effect of ethanol and aqueous extracts of *D. peltata* against Ehrlich's Ascites Carcinoma (EAC) in mice.

MATERIALS AND METHODS

Plant materials

The whole plant of *Drosera peltata* J.E.Sm. was collected from Munnar hills, Kerala, India. It was identified and authenticated by Prof. Madhava Chetty, K., Taxonomist, S.V. University, Tirupati, Andhra Pradesh, India. The plant specimen was deposited to Department of pharmacognosy, Shri Rawatpura Sarkar Institute of Pharmacy (Herbarium specimen no: SRIP/COGNOSY/2011-05). The material was washed, shade dried, powdered and stored in air tight containers for further experiments.

Preparation of the extracts

Alcohol extract: A weighed quantity of the air-dried powdered drug was extracted with ethanol (90 %v/v) in

a Soxhlet apparatus. The extract was concentrated in a rotary flash evaporator at a temperature not exceeding 50°C. The ethanol extract (EEDP) was suspended in distilled water for experimental use.

Aqueous extract: The marc from the ethanol extract was macerated with chloroform- water for 24h to obtain the aqueous extract. This was concentrated under reduced and dissolved in distilled water for experimental studies.

The ethanol (EEDP) and aqueous (AEDP) extracts of *D. peltata* were stored in air tight containers.

Induction of cancer using EAC cells

Ehrlich's Ascites Carcinoma (EAC) cells were supplied by Amala Cancer Research Center, Trissur, Kerala, India. The cells maintained *in vivo* in Swiss albino mice by intraperitoneal transplantation. The tumor cells were injected intraperitoneally (2×10^6 cells per mouse) to animals of all groups except the first group.

Determination of anticancer activity

In anticancer activity study [5], Swiss Albino mice weighing 20-25g were kept in identical laboratory condition and were fed with standard pellet diet and water *ad libitum*. Study protocol was approved by the Institution Animal Ethical Committee (Protocol. No: A. Raju 0903PH2254/JNTUH 2009). They were divided into seven groups viz. Normal group (G1), EAC control group (G2), EAC+ 20mg/kg of 5-Fluorouracil treated group (G3), 250, 500mg/kg of EEDP (G4 and G5) and 250, 500mg/kg AEDP (G6 and G7) of ten each and used for the study. The EAC cells were injected intraperitoneally (i.p, 2×10^6 cells/ mouse) to all groups of animals except G1. On the second day the animals of G3 with 5- fluorouracil (20 mg/kg, i.p), G4 and G5 were treated with 250,500 mg/kg of EEDP and G6 & G7 with 250,500mg/kg of AEDP orally. The treatment was continued for next 14 days. G1 was treated with vehicle.

On day 15, the mice were sacrificed; blood was withdrawn by retro orbital plexus method and the following parameters were checked. The effect of EEDP and AEDP on tumor growth were examined by measuring viable tumor cell count, packed cell volume (PCV), body weight, mean survival time (MST) and percentage increase in life span (%ILS) [6].

Hematological study

On day 15, the mice were sacrificed; blood was withdrawn by retro orbital plexus method and the following parameters were checked, Hemoglobin (Hb), Hematocrit (Hct), RBC, WBC, Neutrophil, Monocyte and Lymphocyte counts[7].

Peritoneal fluid analysis

At the end of the study, inoculated cell line from peritoneal cavity was collected and DNA, RNA, Caspase-3 and total protein were quantified[8].

HPTLC Study

The identification of plumbagin and quercetin in *D. peltata* was determined by High Performance Thin Layer Chromatography (HPTLC) manufactured by CAMAG. Different concentration of standard solution of marker compound (plumbagin and quercetin from Sigma Aldrich) was applied on HPTLC plates along with ethanol and aqueous extract of *D. peltata*. The HPTLC plates were developed in a suitable solvent system and dried in air and scanned at 254 nm. The method was validated in terms of precision and accuracy.

Statistical analysis

The results are expressed as mean \pm S.E.M. The evaluation of the data was done using one way ANOVA followed by Newman-Keul's multiple comparison test; $p < 0.05$ implied significance.

RESULTS

The extracts evoked tumor growth response showed in Table 1 with respect to packed cell volume, viable cell counts and increase in life span. At the doses of 250,500mg/kg of both the extracts, as well as the reference standard drug, 5- fluorouracil, significantly ($p < 0.001$) normalized the body weight and viable cell count when compared with the EAC control group. A similar finding was seen in significant increased in % life span and mean survival time when compared with EAC control group. There was a increased in PCV observed in EAC control mice, upon extract treatment , 500 mg of EEDP showed more significant ($p < 0.001$) than 250 mg/kg of EEDP and 250, 500mg/g of AEDP , where were less significant ($p < 0.01$) in control of PCV. These results indicating that the extracts exhibited a remarkable capacity to inhibit the growth of tumor induced by EAC cell line in a dose-dependent manner in experimental animals.

Table 1: Effect of EEDP and AEDP on body weight, MST, % ILS, PCV, and viable tumor cell count of EAC-bearing mice

| Parameters | Body Weight (g) | MST (Days) | ILS (%) | PCV (ml) | Viable cell count (10 ⁶ cells/mouse) |
|-------------------|------------------------|------------------------|------------------------|-------------------------|---|
| Normal | 22.55±1 | - | - | 14.82±0.2 | - |
| EAC control | 37.98±1.79 | 11.4±0.5 | 28.5±1.28 | 30.56±0.54 | 10.6±1.58 |
| EAC+5FU (20mg/kg) | 22.1±0.89 | 37.8±0.6 | 94.5±1.66 | 18.46±0.25 | 0.76±0.17 |
| EAC+EEDP 250mg/kg | 24.35±1.6 ^a | 35±0.5 ^a | 87.5±1.37 ^a | 25.6±1.5 ^a | 0.44±0.1 ^a |
| EAC+EEDP 500mg/kg | 23.3±0.29 ^a | 36.8±0.86 ^a | 92±2.15 ^a | 27.02±0.65 ^a | 0.32±0.09 ^a |
| EAC+AEDP 250mg/kg | 25.3±0.29 ^a | 35±1 ^a | 87.5±2.5 ^a | 27.3±0.66 ^a | 2.52±0.5 ^a |
| EAC+AEDP 500mg/kg | 24.1±0.7 ^a | 35.2±0.37 ^a | 88±0.94 ^a | 28.01±0.48 ^a | 1.82±0.17 ^a |

The data were expressed as mean± SEM. n = 10. The data analyzed by one-way analysis of variance (ANOVA) followed by Newman-Keul's multiple comparison test.

a- p<0.001, compared to the EAC control group

b- p<0.01, compared to the EAC control group

Hematological effects of the both extracts were shown in Table 2 ,after 14 days of treatment, the hematological parameters of the mice were significantly (p < 0.001) altered, compared to the EAC control group. Total WBC count and Neutrophil count was increased in EAC control group whereas Hb content, RBC count, Hematocrit, monocyte and lymphocyte count were decreased in the EAC control group. 14 days treatment

of both the extracts treatment normalized the altered parameters into more or less normal at the dose of 250,500 mg/kg in which 250mg/kg of AEDP and EEDP showed less significant (p<0.05) on RBC count but in certain parameters 250mg/kg of EEDP and 500mg/kg of AEDP were equal significant (p < 0.01) in certain parameters.

Table 2: Effect of EEDP and AEDP on hematological parameters of EAC-bearing mice

| Parameters | Hb (g %) | Hct (%) | RBC Count (Cells/mLx10 ⁹) | WBC Count (Cells/mLx10 ⁶) | Neutrophil (%) | Mono-cyte (%) | Lymphocyte (%) |
|--------------------|-------------------------|------------------------|---------------------------------------|---------------------------------------|-------------------------|------------------------|-------------------------|
| Normal | 12.46±0.19 | 37.2±2.3 | 5.88±0.16 | 3.78±0.24 | 16.76±0.53 | 1.76±0.12 | 76.46±1.14 |
| EAC control | 7±0.16 | 20.8±0.86 | 2.52±0.21 | 11.42±0.51 | 57.4±1.13 | 0.38±0.06 | 31.4±2.09 |
| EAC +5FU (20mg/kg) | 10.2±0.15 | 37.2±1.65 | 5.42±0.35 | 3.86±0.25 | 16.76±0.14 | 1.44±0.1 | 66.66±1.49 |
| EAC +EEDP 250mg/kg | 11.44±0.16 ^a | 33±1.82 ^a | 4.96±0.13 ^a | 4.74±0.37 ^a | 16.44±0.18 ^a | 1.34±0.12 ^b | 58.46±2.69 ^a |
| EAC +EEDP 500mg/kg | 12.22±0.07 ^a | 33.8±1.07 ^a | 6±0.07 ^a | 4.46±0.36 ^a | 17.52±0.25 ^a | 1.78±0.14 ^a | 74.34±1.06 ^a |
| EAC +AEDP 250mg/kg | 9.74±0.15 ^a | 27.4±1.29 ^a | 4.56±0.2 ^a | 4.2±0.09 ^a | 35.3±1.2 ^a | 0.74±0.11 ^d | 48.66±1.57 ^a |
| EAC +AEDP 500mg/kg | 10.36±0.07 ^a | 30.2±1.74 ^a | 4.94±0.10 ^a | 4.1±0.1 ^a | 26.22±1.32 ^a | 1.06±0.32 ^c | 50.2±1.41 ^a |

The data were expressed as mean± SEM. n = 10. The data analyzed by one-way analysis of variance (ANOVA) followed by Newman-Keul's multiple comparison test

a- p<0.001, compared to the EAC control group

b- p<0.01, compared to the EAC control group

c- p<0.05, compared to the EAC control group

Effect of EEDI and AEDI on peritoneal fluid assay shown in Table 3, increased in DNA, RNA and Total protein and decreased in Caspase-3 level was found in EAC control mice. Treatment with

250,500mg/kg of EEDP and AEDP, significantly (p<0.001) reduced the DNA, RNA and Total protein and increased in Caspase-3 level.

Table 3: Effect of EEDP and AEDP on peritoneal fluid analysis

| Groups | Parameters | DNA(mcg 10 ⁻⁶ cells) | RNA (mcg 10 ⁻⁶ cells) | Caspase-3 (µmol pNA min ⁻¹ mL ⁻¹) | Total protein (mcg 10 ⁻⁶ cells) |
|-----------------------|------------|---------------------------------|----------------------------------|--|--|
| EAC Control | | 8.47±0.15 | 13.9±0.18 | 1.15±0.02 | 119.85±0.9 |
| EAC + 5FU (20mg/kg) | | 4.5±0.15 | 6.13±0.26 | 1.85±0.13 | 40.67±0.95 |
| EAC + EEDP (250mg/kg) | | 4.25±0.13 ^a | 6.65±0.13 ^a | 2.27±0.17 ^a | 39.1±0.92 ^a |
| EAC + EEDP (500mg/kg) | | 3.8±0.08 ^a | 5.33±0.08 ^a | 2.68±0.05 ^a | 35.25±0.24 ^a |
| EAC + AEDP (250mg/kg) | | 5.43±0.13 ^a | 5.5±0.19 ^a | 1.3±0.17 ^d | 52.8±4.78 ^a |
| EAC + AEDP (500mg/kg) | | 4.83±0.17 ^a | 4.6±0.15 ^a | 1.57±0.8 ^c | 40.93±0.26 ^a |

The data were expressed as mean± SEM. n = 10. The data analyzed by one-way analysis of variance (ANOVA) followed by Newman-Keul's multiple comparison test
 a- p<0.001, compared to the EAC control group
 b- p<0.01, compared to the EAC control group

HPTLC study showed that the Rf value of both extracts (EEDP and AEDP) showed a peak at the same Rf value of marker compound plumbagin and quercetin, which confirmed that these two extracts had the

pharmacologically active potential constituents responsible for anticancer effect. The results were shown in Figure 1, 2, 3 & 4.

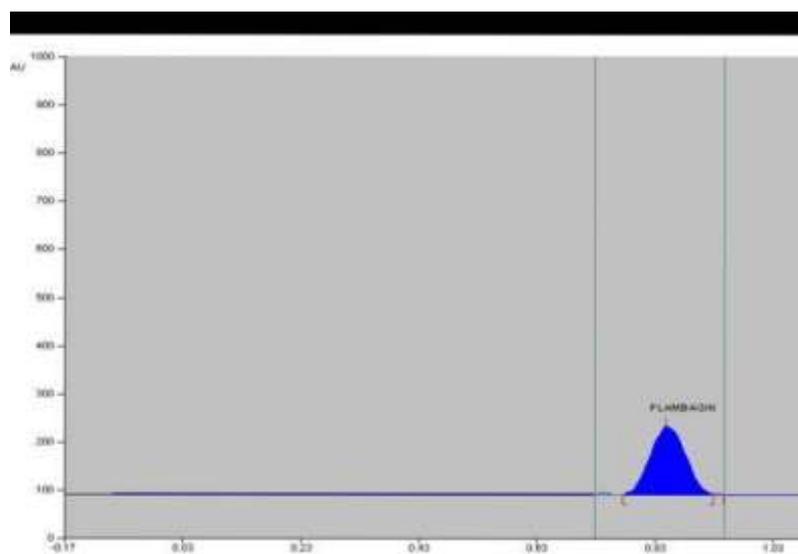


FIGURE 1 - HPTLC chromatogram of EEDP for identification of plumbagin

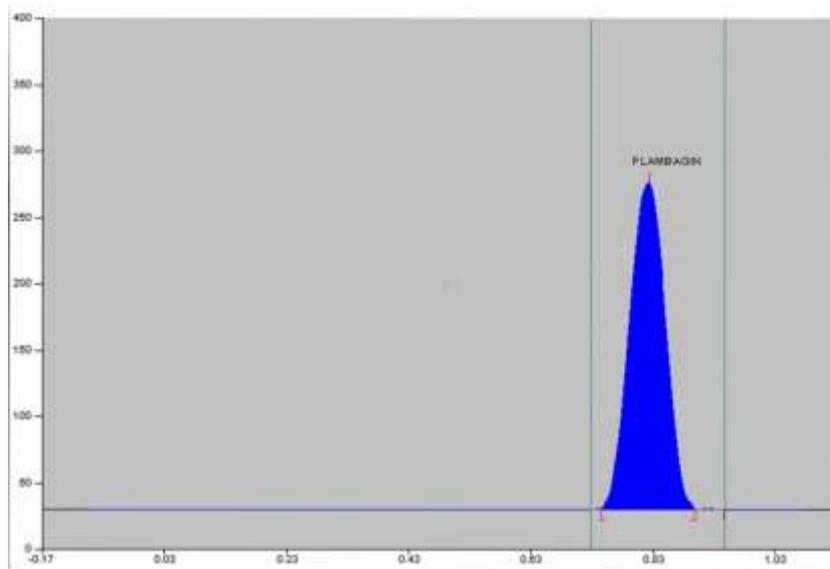


FIGURE 2 - HPTLC chromatogram of marker compound Plumbagin

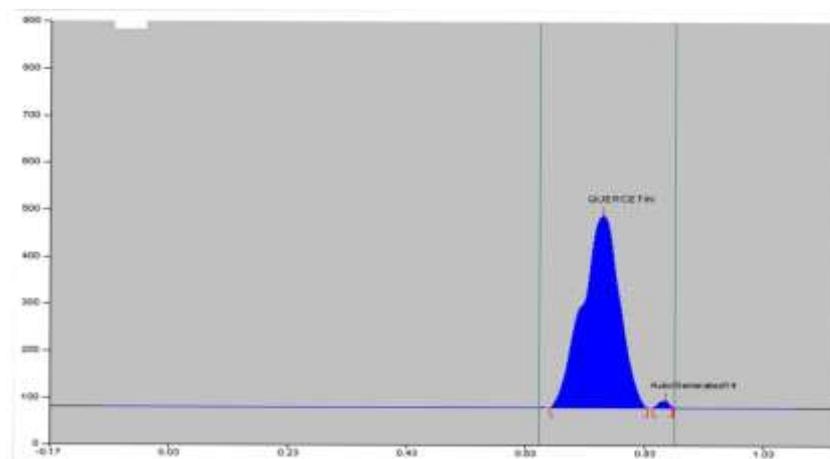


FIGURE 3 - HPTLC chromatogram of AEDP for identification of Quercetin

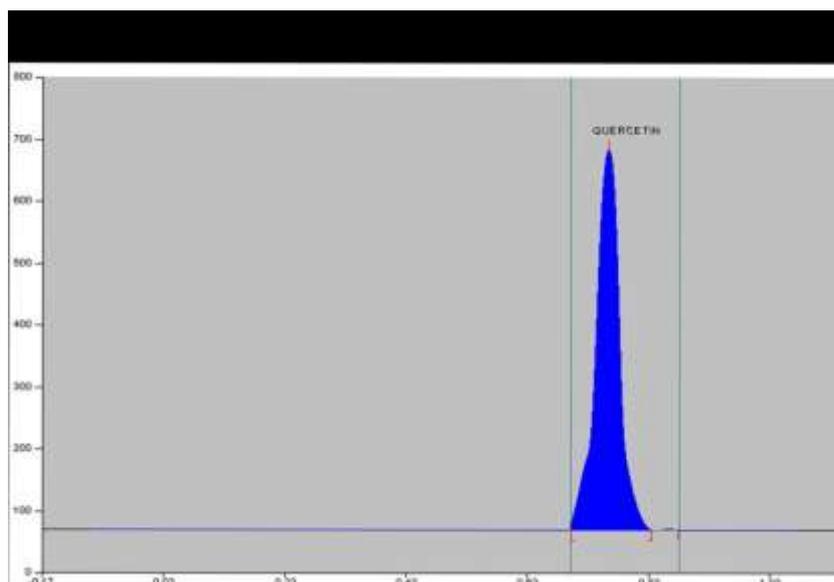


FIGURE 4 - HPTLC chromatogram of marker compound Quercetin

DISCUSSION

Ayurveda- a science of health and longevity has tried many herbal as well as Rasayana remedies with varying degree of success, but its main significance lies in its preventive approach and about 3000 plants, which possess anti-cancer properties and subsequently been used as potent anti-cancer drugs[9]. In the present study, intraperitoneal inoculation of EAC cells in the mice produced an enormous increase in the cancer cell count, which indicated that there is progression of cancer in the animals. The reliable criterion for judging the anticancer effect of plant extract is reduction in viable cell count. It may be due to the extracts stimulate the growth and activity of immune cells by the production of Interleukins, which target tumor cells and cause lysis of the tumor cells by indirect cytotoxic mechanism. Furthermore, the reduced PCV and increased survival time of the mice suggest that the extracts might have exerted a delay in vascular permeability to the cells. The second important criteria for judging anticancer effect is an increased in life span and decrease in WBC count [5].

The reduction in RBC or hemoglobin content and hematocrit (hct) in tumor bearing mice may be due to iron deficiency (anaemia) or due to hemolytic or myelopathic conditions[5]. A low hematocrit is indicated in condition such as anemia, blood loss (traumatic injury, surgery, bleeding colon cancer), nutritional deficiency (iron, vitamin B12, folate) and bone marrow problems. The results showed that both the doses of EEDP and AEDP brought back hemoglobin and RBC count to normal. It also reversed the WBC Count, differential cell count changes in the EAC-bearing mice. This indicates that both the extracts possess protective action on the hemopoietic system.

In order to understand the mechanism of anticancer effect of 250, 500mg/kg of EEDP and AEDP, the main apoptotic marker, Caspase-3 was estimated in the peritoneal cells. Caspases are the central executioners of the apoptotic pathway[8]. They bring about most of the visible changes like cell shrinkage, condensation, margination and fragmentation of chromatin. It also summed up as retention of cytoplasmic organelle structure, but loss of positional interrelationships of organelles. Caspase-3 is particularly activated during apoptosis and its activity was higher in extract treatment groups when compared with EAC control mice. According to Willey, during apoptosis a specific nuclease cuts the genomic DNA between nucleosomes to generate DNA fragments and the presence of this ladder has been extensively used as marker of apoptotic cell death[11]. Hence present study showed that increased Caspase-3 activity decreased DNA, RNA, protein content in the extracts treatment groups. It was strongly proposed that extracts activate apoptotic pathways and implements the anticancer activity on EAC cells.

Generally the major naphthoquinone found in *D. peltata* is plumbagin (2-methyl-5-hydroxy-1,4-naphthoquinone) which increase of apoptotic cells by the activation of caspase-3, which plays a central role in apoptotic process[11]. The main phytoconstituent responsible for the anticancer activity was identified by HPTLC study. Phytochemical standardization is one of the tools for the quality assessment, which includes preliminary phytochemical screening, HPTLC fingerprint analysis and Quantitative analysis of marker compound using modern analytical techniques. In the last few decades (HPTLC) has become known as an important tool for the qualitative semi-quantitative and quantitative phytochemical analysis of herbal drugs and formulations. The major advantage of HPTLC is that several samples can be analyzed simultaneously using a small quantity of marker compound and mobile phase with very less time. The HPTLC chromatogram confirmed that presence of plumbagin and quercetin in ethanol and aqueous extract respectively.

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