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Antitumor Activity of Ethanol and Aqueous Extracts of *Drosera peltata* J. E. Sm against Dalton's Ascites Lymphoma (DAL) in Mice

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ABSTRACT

Background: Plant based drugs are familiar in recent year for the treatment of various ailments including cancer due to safe and economic when compared with modern medicine. Therefore, this present study was aimed to evaluate the anticancer potential of *Drosera peltata* J.E.Sm against Dalton's Ascites Lymphoma (DAL) induced tumor in mice. **Methods:** The ethanol and aqueous extracts of *Drosera peltata* J.E.Sm. were given orally to mice at the dose of 250, 500mg/kg bodyweight for 14 days to DAL 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. **Results:** Treatment caused significant reduction in body weight, packed cell volume (PCV) and viable tumor cell count when compared to the mice of the DAL control group. Restoration of hematological parameters towards normal was also observed. Both the extracts were restored the DNA, RNA, caspase- 3 and total protein content in peritoneal fluid cells upon 14 days oral treatment of plant extracts. 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. **Conclusion:** The results concluded that the ethanol and aqueous extracts of *Drosera peltata*, exhibit significant anticancer activity in DAL- bearing mice.

INTRODUCTION

India is a rich source of medicinal plants, from which natural and derived products such as flavonoids, alkaloids, terpenes and has 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]. One of such a plant is Drosera species and is 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 glucoside rossoliside, flavonoids like quercetin and hyperoside. Plumbagin is 5-hydroxy-2-methyl-1,4-naphthoquinone, a yellow colored pigment found in Plumbaginaceae and Droseraceae[2]. Drosera species are used as vital components in an Ayurvedic preparation called 'Swarna bhasma' (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 Dalton Ascites Lymphoma (DAL) 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 airtight containers for further experiments.

Preparation of the extracts

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 50ºC. The ethanol extract (EEDP) was suspended in distilled water for

experimental use. 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 airtight containers.

Induction of cancer using DAL cells

Dalton Ascites Lymphoma (DAL) cells were supplied by Amala Cancer Research Center, Trissur, Kerala, India. The cells maintained *in vivo* in Swiss albino mice by intraperitoneal (ip) transplantation. The tumor cells were injected ip ($2x10^6$ 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), DAL control group (G2), DAL+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 DAL cells were injected intraperitoneally (i.p, $2X10^6$ 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, 500mg/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. 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]. On day 15, the mice were sacrificed; blood was withdrawn by retro orbital plexus method for haematological studies 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 cells from peritoneal cavity were 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 254nm. The method was validated in terms of precision and accuracy.

Statistical analysis

The results are expressed as mean ± SEM. 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

Anticancer activity

Table 1 shown the tumor growth response which was assessed by calculation of body weight, 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 (5 FU), significantly ($p < 0.001$) normalized the changes occurred in treatment group. Similarly, both the extracts showed significant increased in % life span and mean survival time when compared with DAL control group. There was an increase in PCV in DAL control mice. Treatment with 500, 250 mg/kg mg of EEDP showed more significant ($p<0.001$)than AEDP, to control of PCV. These results indicate that the extracts exhibited a remarkable capacity for inhibition of tumor growth. It was dose-dependent.

TABLE I - Effect of EEDP and AEDP on body weight, MST, % ILS, PCV, and viable tumor cell count of DAL-bearing mice

Parameters	Body Weight (g)	MST (Days)	ILS (%)	PCV (ml)	Viable cell count (10^6 cells/mouse)
Normal	22.5±0.81	40	-	14.82±0.2	
DAL control	35.43±1.34	14±1.14	36±2.81	30.56±0.54	7.16±1.45
DAL+5FU (20mg/kg)	22.63±0.75	36.6±0.9	91.5±2.32	18.46±0.25	0.68±0.12
DAL+EEDP 250mg/kg	23.7±0.8a	35.4±0.5a	88.5±1.28a	25.6±1.5a	0.48±0.11a
DAL+EEDP 500mg/kg	22.1±0.68a	39.4±0.5a	98±0.94a	27.02±0.65a	0.3±0.11a
DAL+AEDP 250mg/kg	26.3±1.03a	32.8±0.86a	82±2.15a	27.3±0.66a	2.54±0.71a
DAL+AEDP 500mg/kg	24.25±0.42a	35±0.55a	86±0.61a	28.01±0.48a	1.9±0.18a

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 DAL control group.

Hematological effects of 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 DAL control group. Total WBC count and Neutrophil count was increased in DAL control group whereas Hb content, RBC count, Hematocrit, monocyte and lymphocyte count were decreased in the DAL 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 DAL-bearing mice

Parameters	Hb (g%)	Hct (%)	RBC Count (10^6/mm^3)	WBC Count (10^3/mm^3)	Neutrophil (%)	Monocyte (%)	Lymphocyte (%)
Normal	12.35±0.31	32.4±0.93	4.55±0.35	4.46±0.24	18.16±0.28	1.82±0.08	76.28±0.48
DAL control	7.12±0.33	18.4±0.68	2.49±0.17	10.44±0.79	58.72±0.81	0.52±0.06	30.48±1.11
DAL+5FU (20mg/kg)	9.2±0.21	35.4±2.18	4.01±0.13	4.46±0.22	16.64±0.27	1.16±0.09	69.14±1.44
DAL+EEDP 250mg/kg	10.8±1.4a	30.8±0.86a	3.21±0.2c	4.52±0.44a	16.76±0.38a	1.1±0.05a	55.3±1.69a
DAL+EEDP 500mg/kg	11.3±0.27b	36.4±1.21a	3.36±0.16b	4.74±0.39a	17.24±0.24a	1.64±0.05a	73.74±2.09a
DAL+AEDP 250mg/kg	9.95±2.1a	26.6±1.33a	3.15±0.3c	3.74±0.38a	32.62±4.79a	0.96±0.08a	54.88±2.45a
DAL+AEDP 500mg/kg	11.71±0.22b	29.6±1.03a	3.65±0.14a	3.92±0.06a	24.06±1.99a	1.14±0.17a	53.84±1.75a

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 DAL control group

b- $p<0.01$, compared to the DAL control group

c- $p<0.05$, compared to the DAL control group

Effect of EEDP and AEDP on peritoneal fluid assay shown in Table 3, increased in DNA, RNA and Total protein and decreased in Caspase-3 level was found in DAL 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

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)
DAL control	8.27±0.14	12.8±0.15	1.13±0.01	114.35±0.89
DAL+5FU (20mg/kg)	4.27±0.13a	6.15±0.32a	1.8±0.07a	41.5±0.44a
DAL+EEDP 250mg/kg	4.5±0.07a	6.23±0.11a	2.13±0.08a	39.87±0.16a
DAL+EEDP 500mg/kg	3.78±0.29a	5.1±0.13a	3.18±0.07a	35.15±0.27a
DAL+AEDP 250mg/kg	5.55±0.18a	6.25±.055a	1.48±0.08b	65.7±1.65a
DAL+AEDP 500mg/kg	5.23±0.24a	4.78±0.69a	1.57±0.11b	40.95±0.55a

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 DAL control group

b- $p<0.01$, compared to the DAL 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. Peak at R_f value of 0.83 represented as standard plumbagin (Figure 2) and the same peak with corresponding R_f value was found with EEDP (Figure 1). Similarly, Peak at R_f value of 0.77 R_f 0.77 was the highest peak generated by standard quercetin (Figure 4) and the same peak at R_f of 0.77 was found with AEDP (Figure 3).

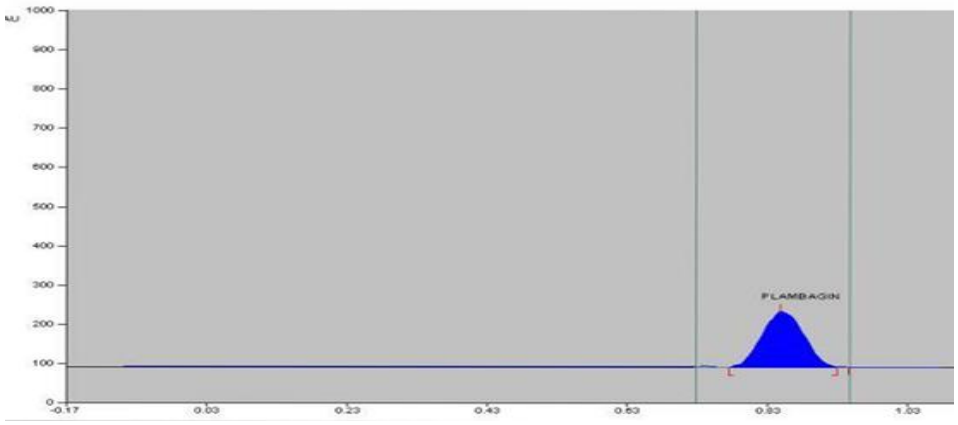

Figure 1 - HPTLC chromatogram of EEDP for identification of plumbagin

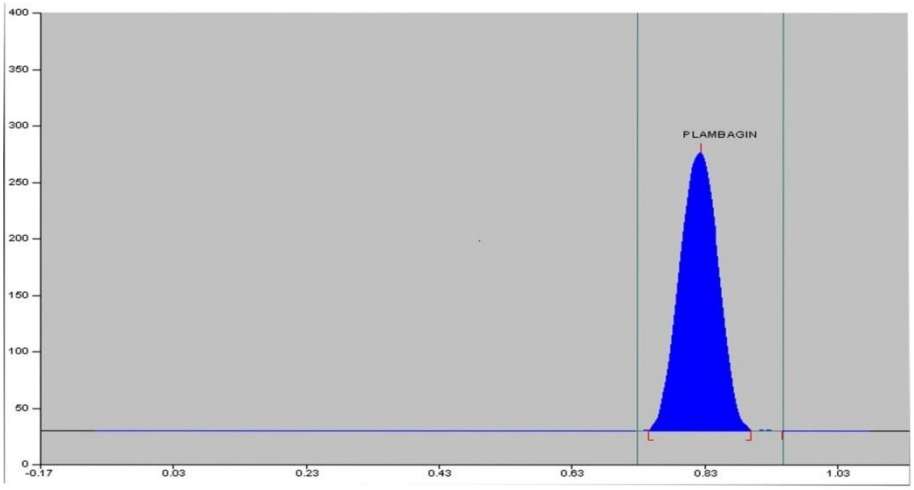

Figure 2 - HPTLC chromatogram of marker compound Plumbagin

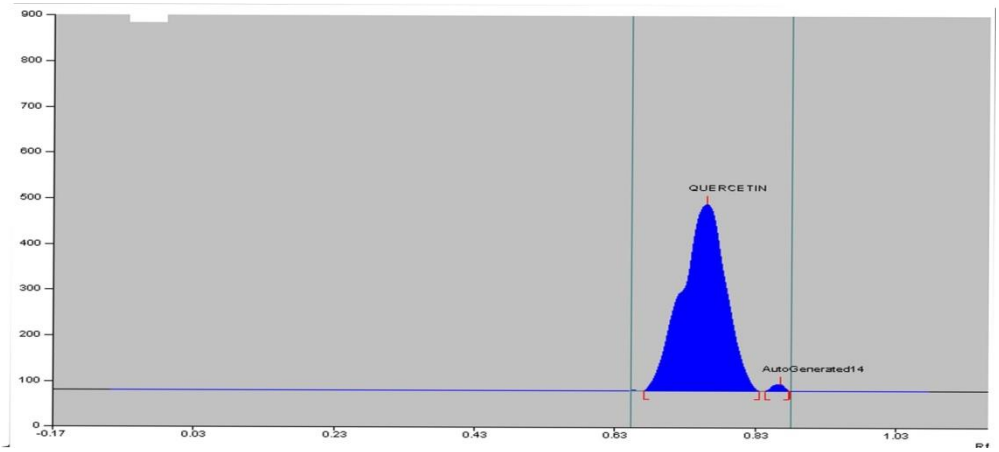

Figure 3 - HPTLC chromatogram of AEDP for identification of Quercetin

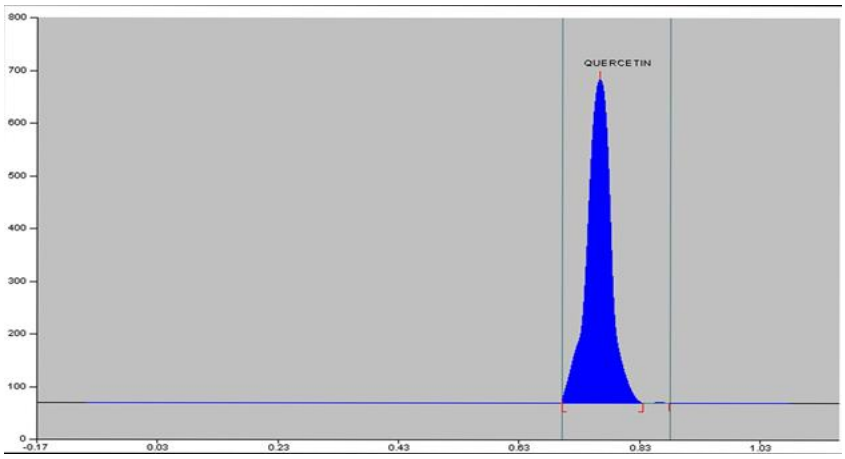

Figure 4 - HPTLC chromatogram of marker compound Quercetin

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 DAL 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 [10].

The reduction in RBC or hemoglobin content and hematocrit (Hct) in tumor bearing mice may be due to iron deficiency (anemia) or due to haemolytic or myelopathic conditions [11]. A low hematocrit is indicated in condition such as anemia, blood loss (traumatic injury, surgery, bleeding colon cancer), nutritional deficiency (iron, vitamin B_{12}, 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 reverses the WBC Count, differential cell count changes in the DAL-bearing mice. This indicates that both the extracts posses protective action on the hematopoietic 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 [7]. 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 [11]. Caspase-3 is particularly activated during apoptosis and its activity was higher in extract treatment groups when compared with DAL control mice. According to Willey [12] 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. Hence present study showed that increased Caspase-3 activity decreased DNA, RNA, protein content in the extracts treatment groups. Our results strongly suggest that extracts activate apoptotic pathways and implements the anticancer activity on DAL 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 [13]. 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.

CONCLUSION

To conclude, the results of the present study demonstrated that the ethanol and aqueous extract of *D. peltata* exhibited antitumor activity against DAL in Swiss albino mice, where the higher dose of the ethanol extracts showed significantly good activity when compared with the lower dose as well as both doses of aqueous extract. The anti-tumor effect of EEDP and AEDP may be due to their antioxidant and free radical quenching properties of the phytoconstituents of these extracts such as plumbagin and quercetin. The possible mechanism

of antitumor effect may be due to stimulation of activate apoptotic pathway mediated through caspase- 3 and its antioxidant effect.

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CONFLICT OF INTEREST

No Conflict of interest.

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