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ORIGINAL ARTICLE

Identity-based High-performance thin Layer Chromatography Fingerprinting Profile and Tumor Inhibitory Potential of *Anisochilus carnosus* (L.f.) wall Against Ehrlich Ascites Carcinoma

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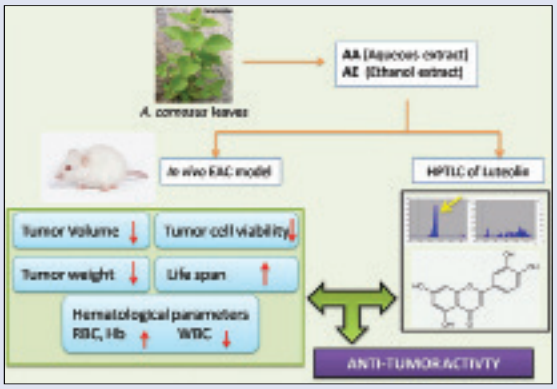
ABSTRACT

Context: *Anisochilus carnosus* (L.f.) wall belonging to the family Lamiaceae is a plant that is widely used in folk medicine for treating eczema, cold, cough, and fever. **Objective:** In the present study, we explored the anticancer potential of *A. carnosus* leaves against Ehrlich ascites carcinoma (EAC) and estimated the quantity of luteolin present in various extracts and fractions of *A. carnosus* by high-performance thin layer chromatography (HPTLC) fingerprinting. **Materials and Methods:** Various factors such as tumor volume, tumor cell viability, tumor weight, prolongation of lifespan, and hematological parameters were assessed. **Result:** We observed a significant lowering in tumor volume, tumor weight, and cell viability in EAC-induced mice following intervention with *A. carnosus* extracts. Also, there was a considerable prolongation of host lifespan and restoration of hematological parameters to almost normal levels with *A. carnosus* treatment. HPTLC fingerprinting of various extracts and fractions of *A. carnosus* along with luteolin as the reference standard revealed the occurrence of luteolin in all tested extracts and fractions of *A. carnosus* with the highest concentration being reported in the ethanol fraction. **Conclusion:** *A. carnosus* exhibits potent anti-tumor potential which can most likely be attributed to the occurrence of different phytochemicals such as phytosterols, terpenoids, and flavonoids in the plant. Further studies to isolate compounds from *A. carnosus* and understand the mechanism of anti-tumor activity would be worthwhile.

Key words: Cytotoxicity, Ehrlich ascites carcinoma, high-performance thin layer chromatography, luteolin

SUMMARY

- EAC induced mice that received *A. carnosus* treatment exhibited significant reduction in tumor volume, tumor weight and tumor cell viability. Their life span was considerably prolonged. We detected luteolin in *A. carnosus* aqueous and ethanol extract using HPTLC. Hence, anticancer activity of *A. carnosus* can be partly attributed to the presence of luteolin.



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INTRODUCTION

Cancer refers to a group of diseases characterized by anomalous cells that can swiftly proliferate, pervade, and metastasize to different vital organs of the body. Death due to this dreaded disease has been on the rise with a probability of almost 5, 89, 430 cancer deaths anticipated to occur in 2015.[1] Although on-going researchers from numerous scientific disciplines are doing their best to find a cure for this malady, the ultimate, perfect one is yet to be discovered.[2] Moreover, patients who undergo the current cancer therapies in the clinical setting go through a number of distressing after-effects such as nausea, hair loss, depression, and constipation[3] making it absolutely essential to find a more effective alternative treatment. Incidentally, plant-based immunomodulators have long been used as a supportive therapy to reduce these distressing side effects and improve the overall health.[4]

Anisochilus carnosus (L.f.) wall (Lamiaceae) commonly known as "Thick leaved lavender" is an annual herb indigenous to the Western Ghats of India. The folkloric benefits of this plant are many. For instance, the tribes in Bhilla district of Maharashtra in India use the roots of this plant for a respite from cold, cough, and fever.[5] The Paliyar tribal community of Tamil Nadu in India smear the leaf paste over the affected regions for treating various skin diseases such as eczema and psoriasis.[6,7] Phytochemically, *A. carnosus* comprises plentiful secondary metabolites such as flavonoids, triterpenoids, phytosterols, tannins, and essential oils[8-10] all of which have tremendous chemopreventive, antioxidant, and anticancer properties.[11-13] In the current study, the anticancer activity of aqueous and ethanol extract of *A. carnosus* has been examined in *in vivo* utilizing the Ehrlich ascites carcinoma (EAC) model. Since luteolin has been acclaimed to have potent anticancer activity, we have endeavored to quantify its concentration in various

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extracts and fractions of *A. carnosus* by high-performance thin layer chromatography (HPTLC) analysis.

MATERIALS AND METHODS

Plant collection

A. carnosus leaves were collected from Udupi in the month of September 2010 and authenticated by Dr. Richard Lobo, Associate Professor, Manipal College of Pharmaceutical Sciences, Manipal, Karnataka, India. It was given voucher number PP 573 and placed in the Department of Pharmacognosy, Manipal College of Pharmaceutical Sciences, Manipal, Karnataka, India.

Extraction

The leaves of *A. carnosus* (300 g) were dried in the shade, coarsely ground, and extracted using soxhlet extractor with ethanol (3 L). The crude *A. carnosus* ethanolic extract (AE) was concentrated under reduced pressure to dryness in a rotary evaporator and stored in a desiccator. For preparation of *A. carnosus* aqueous extract (AA), coarsely powdered leaves (150 g) were macerated in water: Chloroform (99:1). After a week, the extract was strained using a muslin cloth and concentrated on a steam bath and kept in a desiccator. The percentage yield of AE and AA were 12.3% w/w and 16.43% w/w, respectively. Both the extracts were suspended in 0.05% acacia gum (vehicle) for the animal study.

Reagents and chemicals

The reagents sodium chloride, acacia gum, methylene blue, trypan blue, sodium sulfate, methyl violet, and propylene glycol were procured from Merck Specialties Pvt. Ltd., Mumbai, India. Ethanol was bought from Ranbaxy Fine Chemicals Ltd., Sikanderpur, Punjab, India. The standards cisplatin and luteolin were procured from Sigma-Aldrich, US. solvents and chemicals purchased were of analytical grade.

Animals

Eight weeks old Swiss albino mice (20–25 g) were adapted to an investigational room maintained at controlled temperature (22 ± 2°C), humidity (50 ± 5%), and an alternate light and dark phase of 12 h each. They were kept in polypropylene cages with a maximum of two mice per cage and were given water and standard food pellets *ad libitum*. The experiment was performed after attaining ethical clearance from the Institutional Animal Ethics Committee of KMC, Manipal, No. IAEC/KMC/76/2011-2012.

Acute toxicity study and dose calculation

In order to estimate the safe dose for administration, acute toxicity studies were performed by the up and down method in accordance with the OECD Test Guidelines 425.[14] Briefly, the mice were fasted overnight prior to oral administration of *A. carnosus* extracts at various doses (5–2000 mg/kg body weight [b. wt.]). After dosing, they were observed for physical indications of toxicity such as tremors, salivation, convulsion, diarrhea, and coma for the next 1 h, after 4 h, after 24 h, and regularly thereafter for 14 days. Based on these observations, it was noted that AE and AA extracts were safe up to doses of 5 and 1 g/kg b. wt., respectively. Hence, we decided to treat the mice at doses corresponding to 50 and 30 mg/kg b. wt. of AE extract and 50 and 100 mg/kg b. wt. of AA extract.

Tumor model and transplantation

EAC cell lines procured from Dr. Ramadasan Kuttan (Director, Amala Cancer Research Centre, Thrissur, Kerala) were preserved and proliferated in an aseptic environment by serial intraperitoneal (i.p.) transplantation. Using an 18 ml sterile syringe, the ascitic fluid was drawn and checked for ascitic tumor cell viability by trypan blue exclusion assay. In Neubauer's hemocytometer, all cells stained with trypan blue were counted. Once the cells were found to be more than 99% viable, the suspension was diluted with normal saline to obtain a final concentration of 1×10^7 cells/ml. From this stock solution, 0.25 ml was injected i.p. for the formation of a liquid tumor. This was presumed to be day 0 of the experiment.

Experimental design

Swiss albino mice were randomized and split into seven groups ($n = 12$). Animals in every group except Group 1 were EAC induced. Treatment was given consecutively for 13 days in mice belonging to Group 3, 4, 5, and 6. Treatment regimen (via i.p. route) was as follows:

- Group 1: Normal control (0.05% acacia gum)
- Group 2: EAC control
- Group 3: EAC + AE I (30 mg/kg b. wt.)
- Group 4: EAC + AE II (50 mg/kg b. wt.)
- Group 5: EAC + AA I (50 mg/kg b. wt.)
- Group 6: EAC + AA II (100 mg/kg b. wt.)
- Group 7: EAC + cisplatin (3.5 mg/kg b. wt.) on the first day.

Evaluation of anticancer activity

A day following final dosage and 18 h of fasting, six mice in each group was sacrificed by CO_2 exposure following which cervical dislocation was carried out to determine the tumor cell viability, tumor weight, tumor volume, and hematological parameters. Remaining six animals in each group were given water and food *ad libitum* to estimate the prolongation of lifespan in the host.

Tumor weight

Body weight of mice was noted before and after the ascitic fluid was collected from the peritoneal cavity. Weight was expressed in gram (g).

Tumor volume

The volume of ascitic fluid (in ml) was calculated by collecting the ascitic fluid into a graduated centrifuge tube.

Tumor cell count

The tumor cell count was assessed by trypan blue exclusion assay. Briefly, a drop of the ascitic fluid in PBS of was placed on a Neubauer's hemocytometer, and the total number of cells present in the 64 squares was calculated. On staining with trypan blue dye (0.4% v/v in normal saline), cells that were viable did not take up the stain whereas the dead cells were stained blue.

Change in body weight

The weight of all animals was recorded on the day of EAC induction (day 0) and once every 2 days subsequently. The percentage increase in body weight was evaluated by the formula:

Percentage of increase in body weight = (Body weight on the respective day/Body weight on day 0) -1×100.

Hematological parameters

Blood was withdrawn at the end of the investigational period to estimate parameters such as hemoglobin (Hb) content, white blood cell (WBC) count, and red blood cell (RBC) count as per standard guidelines.[15]

Percentage increase in lifespan and mean survival time

The percentage increase in lifespan and mean survival time of the animals in different groups were assessed depending on the mortality of the experimental mice.

$$MST\ (in\ days) = \frac{(Day\ of\ first\ death + Day\ of\ last\ death)}{2}$$

$$\%ILS = \left(\frac{MST\ of\ treated\ group}{MST\ of\ control\ group} - 1\right) \times 100$$

High-performance thin layer chromatography fingerprinting

Preparation of extract and standard

Sample preparation

Two milligrams per milliliter stock solution of *A. carnosus* aqueous and ethanol extracts were prepared by weighing 2 mg of each extract and dissolving in 1 ml methanol.

Standard preparation

One milligram luteolin was weighed and dissolved in 10 ml methanol (analytical grade) to obtain a stock solution having a concentration of 100 µg/ml.

Quantification of luteolin in different extracts/fractions by validated HPTLC method.[16]

HPTLC analysis was performed using Linomat V and CAMAG HPTLC software. Ten microliters of each sample were drawn in a Camag 100 µl syringe and applied onto an HPTLC precoated silica gel G60 F_{254} plate (20 cm × 10 cm) using Linomat V semi-automatic applicator. The chromatogram was developed in a Camag twin trough chamber which was presaturated for 20 min at room temperature with mobile phase constituting toluene: Ethyl acetate: Formic acid in the ratio 10:9:1, v/v/v. The plate was subsequently air-dried and scanned at a scanning speed of 20 mm/s in the absorbance mode at a wavelength of 254 nm and 366 nm using Camag TLC scanner III. The slit dimension was maintained at 6.0 mm × 0.45 mm. The peak areas were recorded, and the percentage of luteolin content in each sample was estimated.

Statistical analysis

Microsoft Office 2010 Beta – Excel was used in graph preparation. GraphPad Prism version 5.0 software (GraphPad Software, Inc., California) was utilized to calculate the IC_{50} via the linear regression equation. Statistical significance (*P* value) was determined using one-way ANOVA followed by Dunnett's *post-hoc* test of significance between treated groups and EAC control group, where $P < 0.05$ was deemed to be significant. Results were denoted as mean ± standard error of the mean ($n = 6$ mice per group).

RESULT

Evaluation of anti-cancer activity

Effect on tumor volume

Tumor volume was estimated in the control and treated groups to gauge the effectiveness of the plant extract in reducing the tumor growth. Several reports have indicated that ascitic fluid is capable of providing nourishment and nutrition to the tumor cells and is vital for their growth and proliferation.[17] Accordingly, in the current study, we noted a build-up in tumor volume in the EAC bearing mice in the control group. However, there was a significant ($P < 0.05$) lowering in tumor volume in the group treated with cisplatin [Table 1]. Likewise, i.p administration of AE II was also able to significantly ($P < 0.05$) cause a decline in tumor volume in Group 3 animals. Similarly, animals in all other treated groups showed comparatively less tumor volume than animals in EAC-induced group.

Effect on tumor weight

EAC-induced group showed a considerable increase in body weight. Administration of cisplatin decreased the elevated body weight significantly ($P < 0.05$). Similarly, *A. carnosus* treatment also reduced the tumor weight in animals at all doses, with a significant ($P < 0.05$) reduction in tumor weight following AA II treatment [Table 1].

Effect on tumor cell count

In the tumor-induced group, we observed more viable and few nonviable cells after staining with trypan blue. In contrast, all treated groups showed a decrease in viable cells. In particular, a significant reduction ($P < 0.05$) was noted in the number of viable peritoneal EAC cells in the AE I, AE II, and AA II treated group [Table 1].

Effect on change in body weight

A substantial increase in body weight of EAC-induced group was noted with the progression of the experiment. There was comparatively less variation in the body weight ($P < 0.05$) in the standard group that received cisplatin treatment. Likewise, in all drug-treated groups, there was less alteration in body weight. A significant difference of $P < 0.05$ and $P < 0.01$was specially noted in AE II, AA I and AE I, AA II treated groups, respectively.

Effect on Hematological parameters

Hematological parameters of treated and untreated groups were evaluated. During tumor progression, there is a usual decline in the level of RBC and Hb[15] as was distinctly established in EAC-induced group [Table 2]. Additionally, a massive increase in the number of WBCs was noted in the same group. We observed that hematological

Table 1: Effect of *Anisochilus carnosus* extracts on tumor growth response

Parameters	EAC	EAC + AE I	EAC + AE II	EAC + AA I	EAC + AA II	EAC + CIS
Tumor volume (ml)	5.8±1.2	3.87±1.3	1.8±0.4*	5.0±0.2	3.1±0.6	0.73±.13*
Tumor weight (g)	6.7±0.13	4.9±1.2	3.1±1.7	4.0±1.2	2.7±0.3*	0.9±0.2*
Viable cell ($\times 10^7$)	7.46±1	3.2±0.7*	2.1±0.9*	5.5±0.18	3.6±0.5*	0.68±0.12*
Nonviable cell ($\times 10^7$)	0.34±0.2	2.6±0.7	4.1±0.7*	2.2±1.3	2.3±0.6	2.22±0.14
Total cell ($\times 10^7$)	7.8	5.8	6.2	7.6	5.9	2.9
Change in b. wt. g)	19.3±2.5	6.3±1.3*	3.3±6.5*	5.9±1.5*	3.7±2.2*	4.2±2.8*
Viable (%)	95.64	55.17	34.8	71.8	55.17	23.44
Nonviable (%)	4.35	44.8	65.19	28.14	44.8	76.55
MST (days)	12.5±0.5	15.5±0.2*	20.5±0.5*	14.5±0.5*	20±0.5*	32±0.7*
% ILS	0	24	64	16	60	156

Values are presented as mean±SEM (n=6 mice per group). Results were analyzed using one-way ANOVA between the treated groups and the EAC control group followed by Dunnett's *post-hoc* test of significance wherein *P<0.05 was considered to be statistically significant. EAC: Ehrlich ascitic carcinoma; AE I: *Anisochilus carnosus* ethanolic extract (50 mg/ml); AE II: *Anisochilus carnosus* ethanolic extract (30 mg/ml); AA I: *Anisochilus carnosus* aqueous extract (100 mg/ml); AA II: *Anisochilus carnosus* aqueous extract (50 mg/ml); CIS: Cisplatin; MST: Mean survival time; % ILS: Percentage increase in lifespan; SEM: Standard error of mean; b. wt.: Body weight

Table 2: Effect of *Anisochilus carnosus* extracts on hematological parameters

Parameters	Normal	EAC control	EAC + AE I	EAC + AE II	EAC + AA I	EAC + AA II	EAC + CIS
RBC (1×10^6 cells/mm^3)	9±0.01	6.05±0.3[a]	7.2±0.8	8.6±1.04	7.9±0.4	9.3±0.04[b,*]	9.8±0.2[b,*]
WBC (1×10^3 cells/mm^3)	5.1±0.3	44±10[a]	6.6±0.2[b,*]	5.5±0.5[b,*]	8.4±2.2[b,*]	4.9±2.1[b,*]	2.8±1.5[b,*]
Lymphocytes (1×10^3 cells/mm^3)	65.0±1.5	38.3±8.2[a]	43.7±0.24	58.4±4.3[b,*]	55.8±0.4[b,*]	60.3±2.1[b,*]	62.3±1.4[b,*]
Monocytes (1×10^3 cells/mm^3)	3.1±0.32	2.4±0.9	3.35±0.2	3.4±0.6	3.5±2.0	3.20±0.1	3.6±0.3
Hb (g/dL)	12±0.16	7.4±1.2[a]	7.9±0.2	10.8±0.7[b,*]	8.6±0.5	11.05±0.5[b,*]	11.3±0.4[b,*]

Values are presented as mean±SEM (n=6 mice per group). Results were analyzed using one-way ANOVA between the treated groups and the EAC control group followed by Dunnett's *post-hoc* test of significance wherein [a]EAC control group versus normal group; [b]All treated groups versus EAC control group; *P<0.05 was considered to be significant. EAC: Ehrlich ascitic carcinoma; AE I: *Anisochilus carnosus* ethanolic extract (50 mg/ml); AE II: *Anisochilus carnosus* ethanolic extract (30 mg/ml); AA I: *Anisochilus carnosus* aqueous extract (100 mg/ml); AA II: *Anisochilus carnosus* aqueous extract (50 mg/ml); CIS: Cisplatin; RBC: Red blood cell; WBC: White blood cell; Hb: Hemoglobin; SEM: Standard error of mean

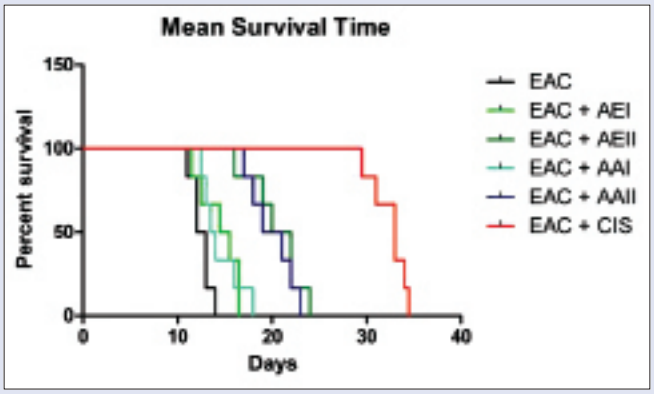

Figure 1: Kaplan–Meier survival plot depicting the mean survival time of *Anisochilus carnosus* extracts treatments

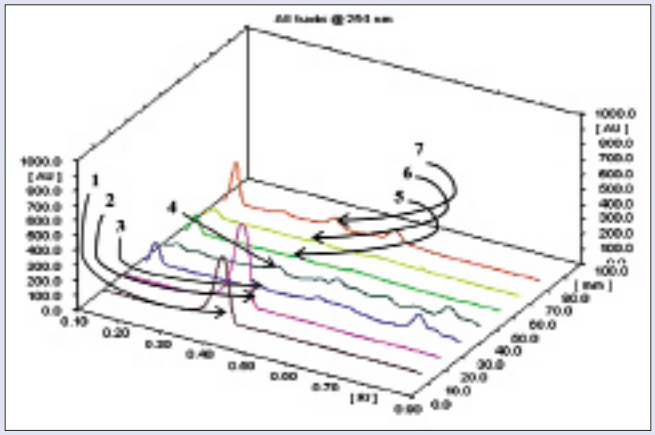

Figure 2: Three-dimensional overview of densitometric chromatogram of standard luteolin and various extracts and fractions of *Anisochilus carnosus* at 254 nm: (1 and 2) Luteolin; (3) Aqueous extract; (4) Ethanol extract; (5 and 6) Aqueous fraction; (7) Ethanol fraction

parameters were brought back to almost normal levels in the treated groups.

Effect on percentage increase in lifespan

Survival rate was higher in the treated group as compared to the animals in the EAC-induced group.

High-performance thin layer chromatography estimation

Quantity of luteolin present in different extracts of *A. carnosus* was estimated by HPTLC. The peak corresponding to luteolin in *A. carnosus* extracts/fractions was identified by comparing with the peak in luteolin reference compound at 254 and 366 nm. It was observed that luteolin eluted at R_f 0.38 at 254 [Figures 1 and 2] and 366 nm [Figures 3 and 4]. Further, we observed that ethanolic fraction contained the maximum concentration of luteolin (0.956% w/w at 254 nm and 1.591% w/w at 366 nm). Tables 3 and 4 list the concentration of luteolin present in different extracts and fractions of *A. carnosus*.

DISCUSSION

EAC originally appeared in the form of murine breast cancer with highly aggressive growth.[18] These cells exhibit traits such as undifferentiation, rapid proliferation, high vascular permeability, and cellular migration which has made it a suitable choice as an experimental model for human tumor.[19] This model is further unique as it can form liquid tumor if the EAC cells are injected intraperitoneally in the mouse and solid tumor if injected subcutaneously.[20] In the present study, various factors such as tumor volume, viable/nonviable tumor cell count, tumor weight, hematological parameters, prolongation of lifespan, and change in body weight were evaluated in animals injected intraperitoneally with EAC cells.

We noted that pharmacotherapy with *A. carnosus* extract significantly reduced the tumor volume in AE I treated group. A substantial increase was observed in the body weight of EAC-induced mice. In contrast, EAC mice that were treated with *A. carnosus* had sufficiently less weight. There was also significant lowering in the viable cell count after treatment with *A. carnosus.* Bone marrow suppression and anemia are identified to be the foremost side effects of chemotherapy.[21] We evaluated the effect of *A. carnosus* extract on hematological parameters of treated and untreated groups. A decline in the level of RBC and Hb usually occurs during tumor progression;[15] we observed a distinct decrease in RBC level in the EAC-induced group [Table 2]. This has been noted and validated in earlier studies.[22] Additionally, a massive proliferation of WBC was noted in the same group. Remarkably, treatment with *A. carnosus* extracts restored the levels of RBC, Hb, WBC, lymphocytes, and monocytes to normal, indicating its protective effect on the hematopoietic system [Table 2]. The ability of an anti-cancer drug to prolong the lifespan of the host is considered to be one of the most dependable benchmarks for evaluating its effectiveness against cancer.[23] *Per se*, we observed a higher survival rate in the treated group as compared to animals in EAC-induced group [Figure 1 and Table 1]. The highlight of the current study is that groups treated with *A. carnosus* extracts revealed remarkable anti-tumor activity even at low doses such as 30, 50, and 100 mg/kg b. wt. In the past, several studies[24-27] have reported good anti-tumor activity *in vivo* with EAC model although with comparatively higher doses of the anti-cancer agent. Also, previous studies on *A. carnosus* have revealed the plant to possess strong anti-tumor activity with its ethanolic and petroleum ether extract exhibiting potent anti-tumor activity *in vitro* against cancer

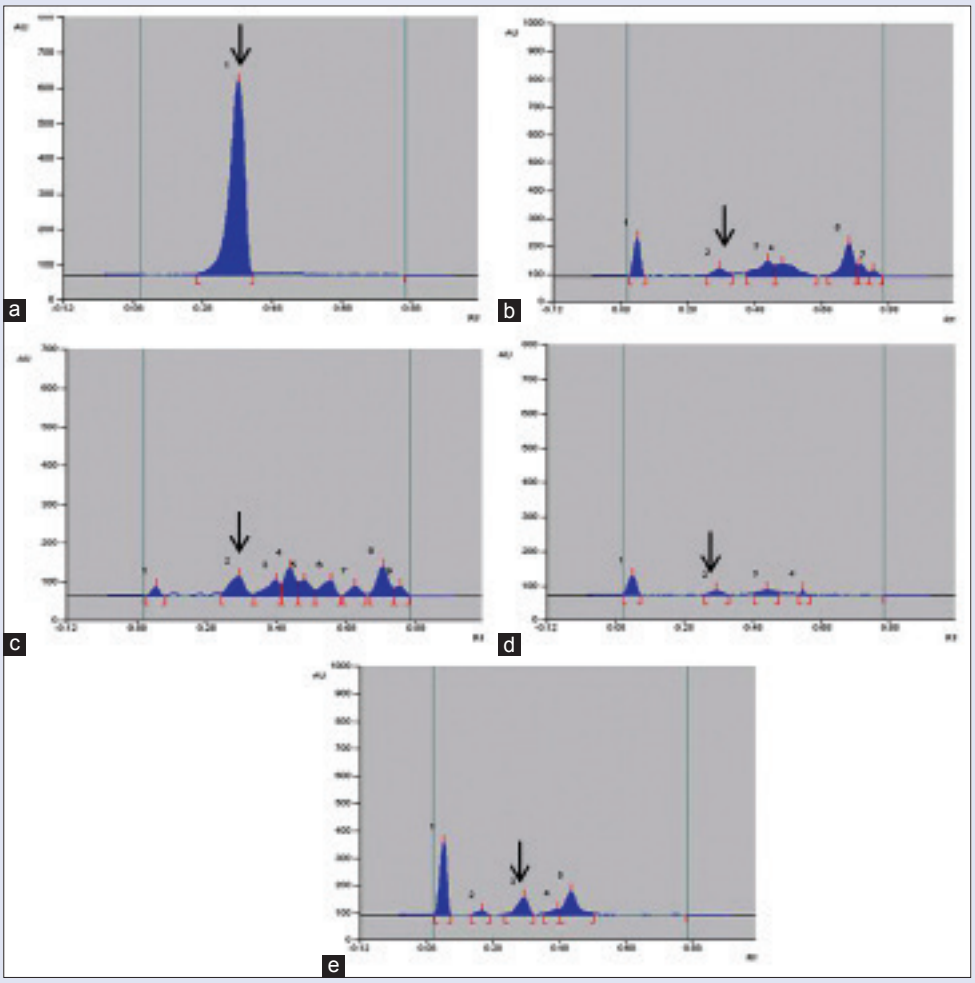

Figure 3: High-performance thin layer chromatography chromatogram of different extracts and fractions of standard luteolin and *Anisochilus carnosus* at 254 nm: (a) Luteolin (100 µg/ml); (b) Aqueous extract (2 mg/ml); (c) Ethanol extract (2 mg/ml); (d) Aqueous fraction (2 mg/ml); (e) Ethanol fraction (2 mg/ml). The black arrow mark indicates the luteolin peak in *Anisochilus carnosus* extract/fraction

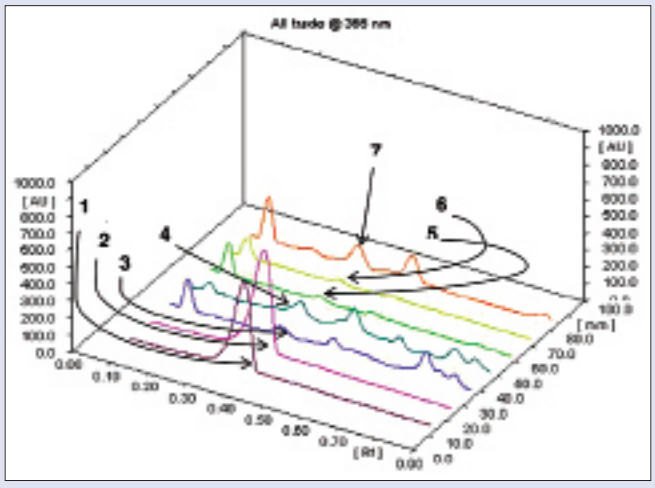

Figure 4: Three-dimensional overview of densitometric chromatogram of various extracts and fractions of *Anisochilus carnosus* at 366 nm: (1 and 2) Luteolin; (3) Aqueous extract; (4) Ethanol extract; (5 and 6) Aqueous fraction; (7) Ethanol fraction

cell lines such as BT-549 (human mammary ductal carcinoma)[28] and A-549 (human alveolar adenocarcinoma).[29]

Traditional medicine has a crucial role to play in cancer therapy, with a multitude of the current anti-cancer drugs being either of natural origin or derived therefrom.[30] Plants are rich in phytoconstituents such as flavonoids, phytosterols, and tannins all of which have exceptional cancer-protective effects. Several clinical trials have indicated flavonoids to be excellent chemopreventive agents.[31] One such naturally occurring flavonoid is luteolin which is commonly found in vegetables such as celery, broccoli, thyme, parsley, carrot, etc.[32] Luteolin is reported to show exceptional anti-cancer activity against several carcinogenic agents such as 7,12-dimethylbenz (a) anthracene, dimethylhydrazine *in vivo*.[33,34] It also exerts potent anticancer activity *in vitro* against numerous malignant cell lines such as MCF-7 (human breast adenocarcinoma), HepG2 (human liver carcinoma cell line), HT-29 (human colorectal adenocarcinoma), OCM-1 (human uveal melanoma) and A375 (human malignant melanoma) etc.[35] Besides, molecular mechanistic studies have indicated that luteolin can bring about the cessation of cell cycle and apoptosis in malignant cells via intrinsic and extrinsic pathways.[36] Since luteolin has been credited with such phenomenal anti-tumor potential, we deemed it worthwhile

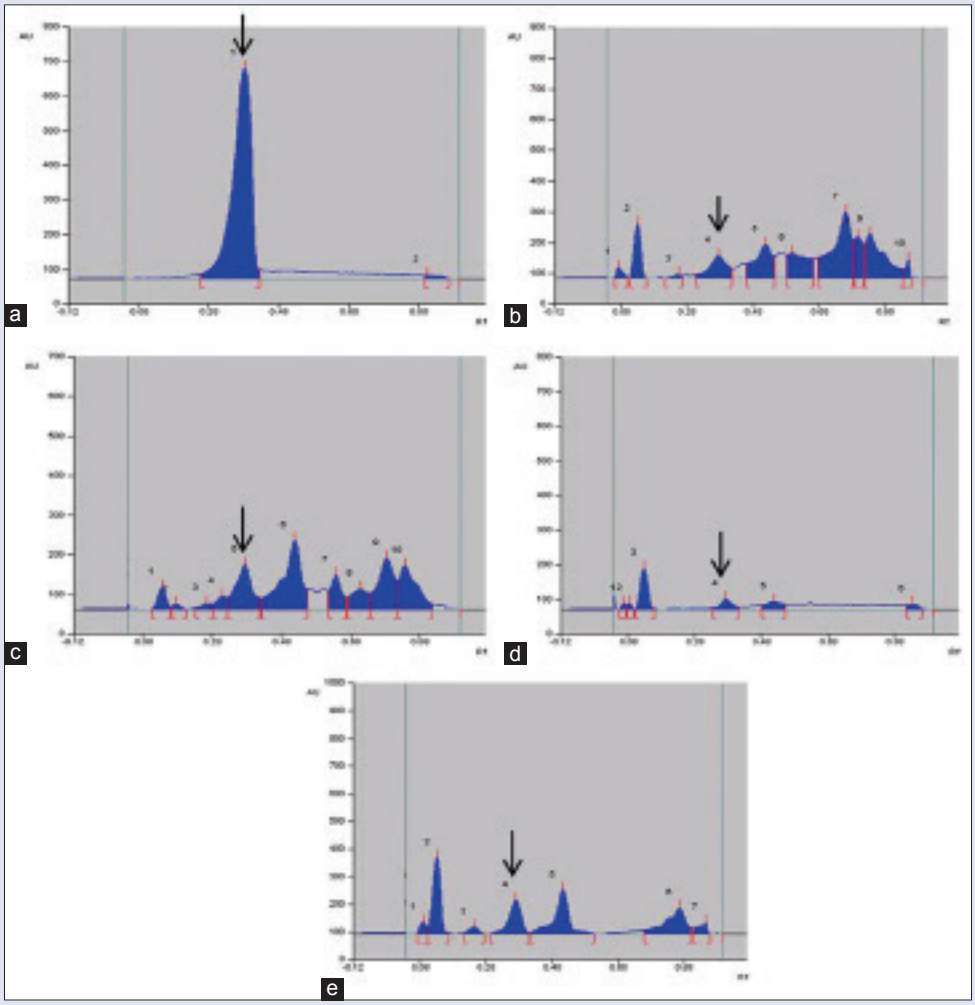

Figure 5: High-performance thin layer chromatography chromatogram of standard luteolin and different extracts and fractions of *Anisochilus carnosus* at 366 nm: (a) Luteolin (100 µg/ml); (b) Aqueous extract (2 mg/ml); (c) Ethanol extract (2 mg/ml); (d) Aqueous fraction (2 mg/ml); (e) Ethanol fraction (2 mg/ml). The black arrow mark indicates the luteolin peak in *Anisochilus carnosus* extract/fraction

Table 3: Estimation of luteolin content in *Anisochilus carnosus* by HPTLC at 254 nm

Fraction	Concentration	R_f	Percentage of content
Luteolin (µg/ml)	100	0.39	100
Aqueous extract (mg/ml)	2	0.38	0.468
Ethanolic extract (mg/ml)	2	0.38	0.854
Aqueous fraction (mg/ml)	2	0.37	0.248
Ethanol fraction (mg/ml)	2	0.38	0.956

R_f: Retention factor; HPTLC: High-performance thin layer chromatography

Table 4: Estimation of luteolin content in *Anisochilus carnosus* by HPTLC at 366 nm

Extract/fraction	Concentration	R_f	Percentage of content
Luteolin (µg/ml)	100	0.39	100
Aqueous extract (mg/ml)	2	0.38	1.223
Ethanolic extract (mg/ml)	2	0.38	1.420
Aqueous fraction (mg/ml)	2	0.37	0.408
Ethanol fraction (mg/ml)	2	0.37	1.591

R_f: Retention factor; HPTLC: High-performance thin layer chromatography

to assess the concentration of luteolin in *A. carnosus* by HPTLC fingerprinting and probe for any possible correlation between its occurrence and anticancer activity. Luteolin content was estimated in various fractions and extracts of *A. carnosus* by HPTLC. The luteolin peak in *A. carnosus* extract/fractions and luteolin reference compound was observed at 254 nm and 366 nm. It was observed that luteolin eluted at R_f 0.38 at 254 nm [Figures 2 and 3] and 366 nm [Figures 4 and 5]. Further, it was noted that ethanol fraction contained the maximum concentration of luteolin (0.956% [w/w] at 254 nm and 1.591% [w/w] at 366 nm) [Figures 2-5, Tables 3 and 4]. Tables 3 and 4 list the % content of luteolin detected in the different extracts and fractions of *A. carnosus*.

CONCLUSION

Considerable increase in lifespan, decrease in tumor volume, tumor weight, and restoration of the hematological parameters to near normal level in *A. carnosus* treated groups in the host could possibly be attributed to the innumerable phytoconstituents present in the plant such as flavonoids, phytosterols, saponins, phenols, tannins, and volatile oil. HPTLC fingerprinting confirmed the presence of luteolin in various extracts and fractions of *A. carnosus*. As such, luteolin in *A. carnosus* may have a crucial role to play in tumor suppression of EAC. Efforts toward identification and isolation of other compounds from *A. carnosus* specifically responsible for anti-tumor activity would be worthwhile.

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Conflicts of interest

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