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International Journal of Pharmaceutical Sciences and Drug Research

[ISSN: 0975-248X; CODEN (USA): IJPSPP]

Available online at www.ijpsdronline.com

Research Article

In vitro and *In vivo* Anti-Cancer Efficacy of *Adiantum capillus-veneris* L. against some selected Human Cancer Cell Lines and on Ehrlich Ascites Carcinoma Mouse Model

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ARTICLE INFO

Article history:
Received: 26 February, 2020
Revised: 26 April, 2020
Accepted: 01 May, 2020
Published: 30 May, 2020

Keywords:
Adiantum capillus-veneris L.,
Antitumor, Cancer,
Ehrlich ascites carcinoma,
In vitro, In vivo.
MTT assay.

DOI:
10.25004/IJPSDR.2020.120309

ABSTRACT

Adiantum capillus-veneris, commonly known as maidenhair fern, belongs to family Pteridaceae, has traditionally been used in various medicinal preparations as demulcent, expectorant, emmenagogue, diuretic, etc., in the form of oil, paste, decoction, and powder. It also has prominent role in hair growth and has anti-microbial, anti-inflammatory, anti-diabetic, anti-nociceptive, and antioxidant properties of therapeutic interest. This study aimed to investigate the *in vitro* cytotoxic activity of fractions of ethanolic extract isolated from the aerial part of *A. capillus-veneris* against some human cancer cell lines, such as, colon (HCT-116), lung (A549), breast (MCF-7), and pancreatic (MIA PaCa-2) and tumor cell proliferation/ inhibition was assessed using MTT assay. The *in vivo* anti-cancer activity of hexane fraction was also evaluated against murine Ehrlich ascites carcinoma (EAC) model. The results confirmed that all the fractions of ethanolic extract exhibited promising *in vitro* inhibition of tumor cell proliferation when tested against different human cancer cell lines. Among all, hexane fraction proved to be more effective, having IC_{50} values 21.72, 22.67, 26.25 µg/mL, for HCT-116, A-549, MCF-7, respectively, but chloroform fraction revealed to be more cytotoxic against Mia-PACA-2 having IC_{50} value 14.72 µg/mL. Higher cytotoxic activity is found to be associated with lower IC_{50} values. The findings showed that all five fractions exhibited dose-dependent killing capabilities in various human-derived cancer cell lines at 48 hours of treatment. Hexane fraction was found to inhibit tumor growth development by 16.95, 41.12, and 82.07% at 50, 100, and 200 mg/kg body weight, respectively. Additionally, this fraction was predicted to be non-toxic at the tested doses. The findings indicate that *A. capillus-veneris* herb is an antineoplastic agent and suggests that further studies evaluating the isolation of active antitumor compounds from *A. capillus-veneris* and their mechanism(s) of action are necessary.

Introduction

The global use of natural products, such as, plants and plant products, has become more essential in primary health care needs, particularly in developing countries. Various pharmacological and pharmacognostical analyses were carried out to discover novel bioactive compounds or to find out new configurations for the improvement of novel remedial agents for the treatment of human ailments, like cancer and other infectious diseases.[1] Since the beginning of human civilization, plants have been in use for medicinal purposes and became the sources of current medicine. Largely chemotherapeutic drugs used against cancer were recognized and isolated from plants or their synthetic derivatives.

Cancer is known to be one of the major causes of death throughout the world. At global scale, the morbidity of cancer is raised, signifying that the existing cancer strategies, such as, chemotherapy, radiotherapy, surgery, etc., have to be synchronized by novel anti-cancer representatives with advanced efficacy and less toxic

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Relevant conflicts of interest/financial disclosures: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

effects. Concurrently, in developing countries, these must be accessible at affordable costs for patients. In this regards, medicinal plants and their products have become prominent resources.[2] Taking into consideration, chemotherapy became unsuccessful in a huge number of patients having cancer, the improvement of drug resistance is rather a challenge for a successful organization of cancer.[3] In many developing countries, herbal medicine plays a chief role in health care due to their rich customary awareness and extensive accessibility.[4]

A. capillus-veneris L. also known by southern maidenhair fern belongs to the family Pteridaceae is an herbaceous perennial fern which is most common and widely distributed throughout the world. In the *Ayurvedic* system of medicine, the genus is popularly known as *"Hansraj"*.[5] Ethno-medicinally, this genus has been used against different fungal and bacterial strains, which suggests that it exhibits anti-microbial activity.[6] Commonly this genus has been used in the treatment of inflammatory disorders, including bronchitis, gastritis, cystitis, nephritis, and dermatitis.[7,8] It has been reported that by inhibiting NF-κB activation, this fern hinders the inflammatory mediator's production.[9] It has also been reported that this fern has been used as a tonic and diuretic in the treatment of hepatitis, jaundice, cold, cough, fever, and bronchial chaos, as a demulcent, stimulant, purgative, emollient, general tonic, and hair tonic. In addition to skin diseases, this fern is reported to be effective against tumors of liver, spleen, and other viscera.[6]

Concerning the phytoconstituents of *A. capillus-veneris*, reveals the presence of sterols,[10] sulfate esters of hydroxycinnamic acid–sugar derivatives,[11] flavonoids, such as, quercetin, quercetin-3-O-glucoside, querciturone, quercetin-3-O-rutinoside (rutin), nicotiflorin, kaempferol-3-sulfate, procyanidin, astragalin, isoquercitrin, populnin, prodelphinidin, and naringin. A number of triterpenoids, including oleanane triterpenoid compounds, were also reported to be present in *A. capillus-veneris*.[12-14]

Moreover, *A. capillus-veneris* has been utilised as antioxidant,[15] anti-inflammatory agent,[9] anti-nociceptive,[16] anti-diabetic,[17] antimicrobial[18] agent, etc. On the basis of a variety of pharmacological activities, the present investigation deals with the pre-extraction processes, extraction with ethanol and fractionation of ethanol extract using various solvents, such as, hexane, chloroform, ethyl acetate, and n-butanol, including water fraction and aimed to evaluate the anti-cancer potential of all the fractions derived from *A. capillus-veneris in vitro,* as well as, investigation of the anti-cancer potential of hexane fraction on the experimental animal model *in vivo*. As a novelty of the presented work, the anti-cancer work on *A. capillus-veneris* is not much more investigated and is almost unexplored.

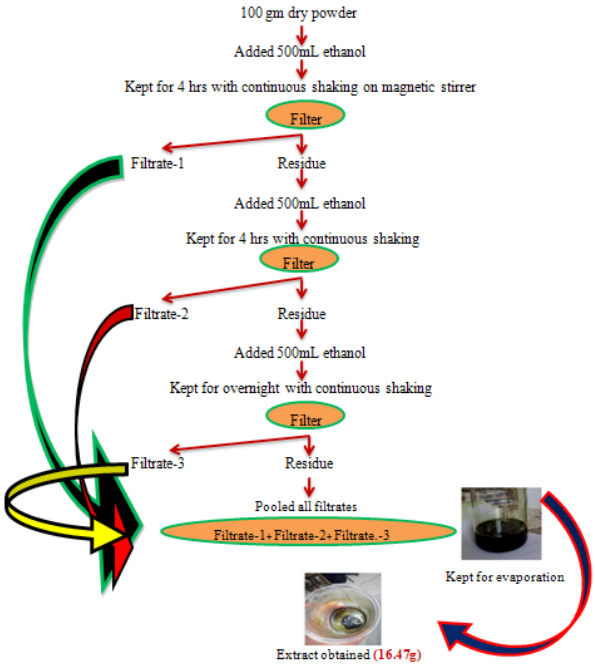

Fig. 1: Extraction by maceration technique

Materials and Methods

Chemicals and Reagents

The RPMI-1640 medium, fetal calf serum (FCS), trypsin, gentamycin, penicillin, 3-(4,5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT), dimethyl sulphoxide (DMSO), were purchased from Sigma-Aldrich (St. Louis, MO, USA). Ethylenediaminetetraacetic acid (EDTA), tris-base, phosphate-buffered saline (PBS), 5-FU, NaCl were procured from HiMedia Laboratories Pvt. Ltd., Mumbai, India.

Plant Preparation and Extraction

Healthy, disease-free plants of *A. capillus-veneris* were collected from the Hirpora region of the Shopian forest above 150 to 200 meters from the ground level, Shopian, Jammu and Kashmir, in June 2016. The average temperature of the site of the collection was 27 ± 2°C. The authenticity was confirmed by Dr. Akhter Husain Malik, Curator, Centre for Biodiversity and Taxonomy, Department of Botany, University of Kashmir. Voucher sample (specimen no. 2427-KASH) was also prepared and kept in the herbarium of the Department of Botany, University of Kashmir, Jammu and Kashmir, India.

The aerial parts of the fern *A. capillus-veneris* were removed, cleaned, washed to remove soil, mud, and other adhering material, and were then air and shade dried, and powdered using kitchen milling mechanical grinder. The powdered sample was then extracted by maceration (Fig. 1) method, using ethanol as solvent. The extract was then dried under vacuum at a temperature. The yield of the ethanolic extract was (16.47% w/w). The extract was mixed with water, and then fractionated by different

solvents (Fig. 2) of increasing polarity using hexane, chloroform, ethyl acetate, and n-butanol. Finally, five fractions were obtained. The yields of hexane, chloroform, ethyl acetate, n-butanol, and water were 48, 9, 11, 18, and 20.5% (w/w), respectively.

Cell Culture and Treatment

Four human cancer cell lines, such as, colon (HCT-116), lung (A549), breast (MCF-7), and pancreatic (MIA PaCa-2), were obtained from CSIR-Indian Institute of Integrative Medicine, Jammu. The cell lines were routinely cultured in RPMI-1640 growth medium. The growth media (pH 7.2) was supplemented with 10% FCS, 1% penicillin (100 units/mL), and streptomycin (100 µg/mL) in tissue culture flask in an incubator at 37°C with 95% relative humidity and 5% CO_2 gas environment. The cells were harvested by trypsinization. Stock solutions (1 mg/mL) of all the fractions were dissolved in DMSO and diluted with complete growth medium containing 50 µg/mL of gentamycin to the desired concentrations [0 (control), 10, 25, 50, 100, and 200 µg/mL]. Untreated control culture received only the vehicle (DMSO < 1%).

In vitro Cytotoxicity Screening through MTT Assay

The cytotoxicity of different fractions of ethanolic extract secluded from *A. capillus-veneris* aerial part were assessed against HCT-116, A549, MCF-7, and MIA PaCa-2 cell lines through the tetrazolium salt or MTT assay.[19] These human carcinoma cell lines allow the evaluation of the cytotoxicity of plant products *in vitro*. The MTT assay is based on enzymatic reduction of the yellow-colored water-soluble MTT dye to purple-colored formazan crystals by variety of mitochondrial dehydrogenase enzymes (Fig. 3) that are functional in viable cells. The reduction product is a water-insoluble blue formazan that can then be dissolved in DMSO for colorimetric measurement.

All the cell lines were seeded at the density of 1×10^5 cells per well in a 96-well plate with 100 µL of growth medium. After overnight growth, cells were incubated with various concentrations such as 0 (control), 10, 25, 50, 100, and 200 µg/mL of all the fractions. Four hours prior to the completion of 48 hours of treatment of these fractions, 20 µL of freshly prepared MTT solution (2.5 mg/mL in PBS) was added to each well and re-incubated for 4 hours at 37°C. After 48 hours, the culture medium containing MTT was removed, and formazan crystals were solubilized by the addition of 100 µL of DMSO to each well. Absorbance (OD) was then assessed at 570 nm using an absorbance microplate reader (Bio-Rad microplate reader). The cell viability was calculated as the percentage of viable cells that produced dark blue formazan product in the treated cells compared to the control cells, and the percentage cell growth inhibition was calculated as:

Percent cell growth inhibition = 100-cell growth viability

The half-maximal inhibitory concentration (IC_{50}) was calculated using Graph-Pad Prism software. Three independent experiments were performed with similar results; representative results of one experiment are presented.[20]

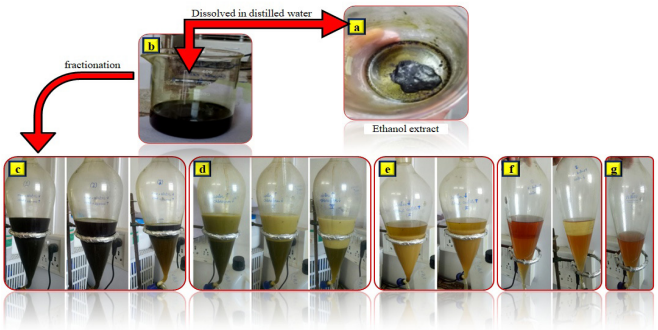

Fig. 2: Fractionation of ethanol extract; (a) by various solvents; (b) ethanol extract dissolved in distilled water; (c) hexane; (d) chloroform; (e) ethyl acetate; (f) n-butanol; (g) water

Acute Oral Toxicity Study

Concerning the cytotoxicity of all the fractions of ethanolic extract of *A. capillus-veneris*, almost hexane fraction proved to be more effective than other fractions. Before employing this fraction for the *in vivo* experiments, it has been used to evaluate whether this fern is risk-free or not. Therefore, hexane fraction was evaluated for acute oral toxicity in mice following the recommendations of the Organization for Economic Co-operation and Development (OECD, 2001) to study the acute toxic effects and to determine the minimum lethal dose of extracted drug.

Selection of Animals

One week acclimatized BALB/c mice of either sex, weighing between 23 to 29 grams were used. All the mice were housed under 12 hours light and 12 hours dark well-ventilated room having temperature 23 ± 2°C. Animals were fed with free access to food and water *ad libitum*. The procedure to find the oral toxicity of the isolated fraction was permitted by the institutional animal ethical committee (IAEC) according to the assistance of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA).

Method

The animals were divided into six groups; each group contained five mice. Hexane fraction was tested using a single amount a day at the dose of 50, 100, 300, 500, and

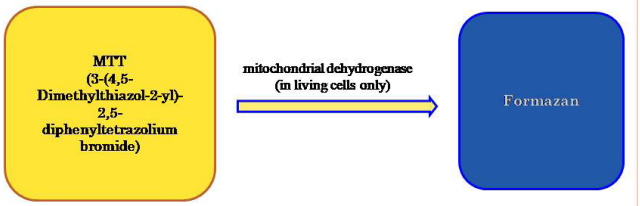

Fig. 3: Reduction of MTT to produce formazan

700 mg/kg body weight to animals of groups II, III, IV, V, and VI, respectively. Mice of group I served as the control, and received only vehicle. Before dosing, all the animals were subjected to fasting along with water overnight (16–18 hours). Each mouse received test formulations orally by gavages. Animals were fed on a normal diet after 3 to 4 hours of dosing.[21] After the administration of the hexane fraction, the animals were observed continuously for 14 days, for any toxic demonstration. The observations were based on the changes in the skin, mucous membranes, eyes, fur, and also their respiratory, circulatory, and behavioral patterns. Interest was also given towards whether the animal has tremors, convulsions, diarrhea, sleep, and comma.

In vivo Antitumor Activity

The hexane fraction of the ethanolic extract of *A. capillus-veneris* aerial part was investigated for *in vivo* anti-cancer activity against EAC murine model of mice.

Experimental Animals

Healthy female BALB/c mice weighed in the range of (23 to 29 grams) were obtained from animal house, CSIR-Indian Institute of Integrative Medicine, Jammu. The animals were housed in propylene cages and maintained under the controlled conditions at temperature 23 ± 2°C, constant photoperiod (12 hours light/ 12 hours dark), and relative humidity 50–60%. The room was ventilated with 100% fresh air. Animals were fed with standard pellet diet (M/s Ashirwad Industries, Chandigarh, India), and autoclaved water *ad libitum* was provided. Good concern was taken to preserve them in a well form and to avoid any menace of possible pathogenic contaminations. The experimental protocol used in this study was approved by the Institutional Animal Ethics Committee, CPCSEA, of Dr. Harisingh Gour Vishwavidyalaya, a Central University, Sagar, M.P., India (approval no. 379/CPCSEA).

Induction of EAC in Experimental Groups of Animals

The EAC cells were obtained from CSIR-Indian Institute of Integrative Medicine, Jammu, which were maintained *in vivo* in BALB/c mice having an 8 to 10 days old ascetic tumor. The EAC cells were collected from peritoneal cavity of the mice using saline while transforming the tumor cells to the experimental animals. The cells were counted using haemocytometer, diluted to the appropriate concentration, and then 1 × 10^7 EAC cells per mouse were inoculated intraperitoneally in all (n = 38) BALB/c female mice by using a sterile disposable syringe under aseptic conditions.[22] On day 1, mice were randomized and estranged into five groups. Four treatment groups contained seven animals each, and one control group contained ten animals. The schematic representation for the experiment is shown in Fig. 4. Treatment was given as follows:

Group I: 0.2 mL of normal saline; i.p. injected in mice having EAC (1 × 10^7 cells/mouse) from day 1 to 9.

Group II: Consecutively injected with hexane fraction (50 mg/kg i.p.) once a day in mice having EAC (1 × 10^7 cells/mouse) from day 1 to 9.

Group III: Injected consecutively with hexane fraction (100 mg/kg i.p.) once a day in EAC induced mice from day 1 to 9.

Group IV: Injected consecutively with hexane fraction (200 mg/kg i.p.) once a day in EAC induced mice (1 × 10^7 cells/mouse) from day 1 to 9.

Group V: 20 mg/kg i.p. body weight having EAC (1 × 10^7 cells/mouse), 5-flurouracil injected consecutively from day 1 to day 9.

The first group was the tumor-bearing control administered with normal saline (0.2 mL, i.p.); the second, third, and fourth groups served as treatment groups, and the fifth group served as positive control treated with 5-fluorouracil. On day 12, all animals were weighed and then sacrificed. The ascitic fluid was collected from the peritoneal cavity of each mouse in graduated tubes for the quantification of tumor weight and volume. Tumor cells were counted on haemocytometer (the whole process is shown in Fig. 5). The percent tumor growth inhibition was calculated by comparing the total number of tumor cells present in the peritoneal cavity of treated groups and the

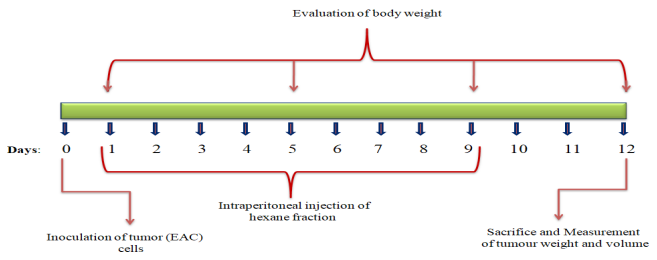

Fig. 4: Schematic representation of the experimental plan for EAC in BALB/c mice

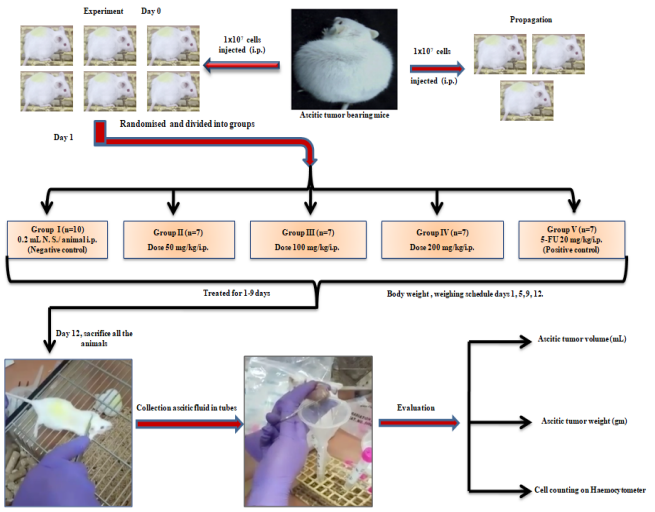

Fig. 5: Method used for the collection of ascetic fluid from EAC induced mice

control group as on day 12 of the experiment. Tumor cell growth in the saline-treated control group was taken as 100 percent cell growth.

$$\% \text{ tumor growth inhibition} = \frac{\text{Average no.of cells in control} - \text{Average no.of cells in treated animals}}{\text{Average no.of cells in control}} \times 100$$

Results and Discussion

The preliminary step in medicinal plant studies is based on the preparation of plant samples in order to preserve the secondary metabolites in the plants before extraction. In the present investigation, the solvent extraction of aerial part of *A. capillus-veneris* was carried out via the maceration method using ethanol as solvent. The best advantage of use of ethanol as solvent is that it extracted out polyphenolic substances and is safe for human consumption.[23]

In vitro Antitumor Activity through MTT Assay

Since prehistoric times, concern regarding the pharmacological consequences of bioactive components towards cancer prevention and treatment has improved significantly. Plants or the plant products utilized in traditional remedies have contributed novel bioactive substances towards the field of anticipatory and therapeutic medicines. Plant-derived bioactive components represent a universal choice for the anticipation of cancer all over the world, and these bioactive components became the primary causes for the improvement of antitumor drugs used clinically.[24] They have been shown to acquire abundant anti-cancer properties against a variety of cancer cells by means of varied forms of cytotoxic effects devoid of demonstrating substantial damage to normal cells.[25]

Keeping in view the anti-cancer potential of the fractions of ethanol extract, the present study involves the *in vitro* anti-cancer efficacy of different fractions of ethanol extract of *A. capillus-veneris* against four human cancer cell lines. Consequently, the current research was snooping out the *in vivo* anti-cancer potential of hexane fraction in an EAC induced carcinoma in five groups of animals.

Different cell lines of different origin showed unlike results towards the isolated fractions of ethanolic extract of *A. capillus-veneris*. Hence, it is essential to take more than one cell line for the initial screening experiments. To evaluate the cytotoxic activity of different fractions of ethanol extract isolated from aerial part of *A. capillus-veneris*, four human cancer cell lines such as colon (HCT-116), lung (A549), breast (MCF-7), and pancreatic (MIA PaCa-2) were incubated with different doses from 10 to 200 µg/mL of all fractions. After 48 hours of incubation, cell viability was determined by MTT assay. Concerning the cytotoxicity of all the fractions (hexane, chloroform, ethyl acetate, n-butanol, and water fractions) of ethanolic extract of *A. capillus-veneris* against HCT-116, A549, MCF-7, and MIA PaCa-2 cells at different concentrations such as 0, 10, 25, 50, 100, and 200 µg/mL revealed cell cytotoxicity in a concentration dependent. *In vitro* screening of these fractions showed that all the fractions of ethanolic extract of *A. capillus-veneris* were proved to be cytotoxic against these four human cancer cell lines.

The cytotoxic effect of all the fractions were observed in the following order hexane > ethyl acetate > chloroform > water > n-butanol (HCT-116), hexane > chloroform > water > ethyl acetate > n-butanol (A549), hexane > chloroform > ethyl acetate > water > n-butanol (MCF-7) and chloroform > hexane > ethyl acetate > water > n-butanol (MIA PaCa-2).

Furthermore, IC_{50} values of all the fractions were calculated against these cell lines and are depicted in Fig. 6. Higher anti-cancer activity was found to be associated with lower IC_{50} values. Among all fractions, hexane fraction showed higher cytotoxic effect at 48 hours of treatment against HCT-116, A549, and MCF-7, but for MIA PaCa-2 cancer cell line, chloroform fraction was more effective.

Acute Oral Toxicity Assay

No deaths occurred throughout the 14 days of the study period by the administration of hexane fraction at different doses from 5 to 700 mg/kg body weight. Physical observations of the hexane fraction treated mice indicated that none of them were reported any signs of toxic effects such as, changes in the skin, mucous membrane, eyes and fur, respiratory, circulatory and behavioral patterns, tremors, convulsions, diarrhea, sleep, and comma. Overall, the study showed that this fraction was well tolerated by the mice for a maximum dose level of 700 mg/kg body weight. Based on this observation, dose level of 50 to 200 mg/kg body weight of this fraction was selected for other *in vivo* pharmacological studies.

In vivo Antitumor Efficacy

The EAC is an expected murine mammary adenocarcinoma personalized to ascites form and is transmitted in outbred mice by consecutive intraperitoneal (i.p.) routes. In any mouse host, Ehrlich ascites tumor cells proliferate in a rapid way because of the absence of H-2 histocompatibility antigens, while for the depiction of Ehrlich ascites, various experimental studies were developed to use

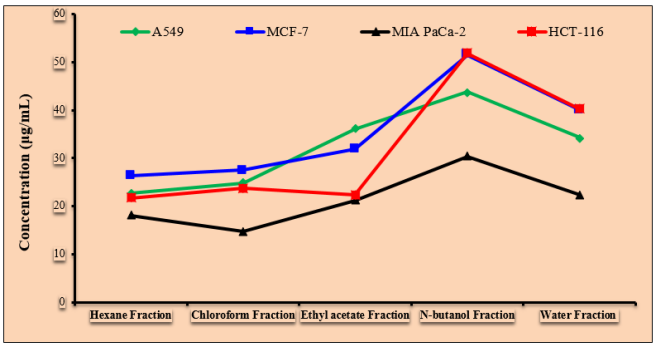

Fig. 6: IC_{50} values of all the fractions of alcoholic extract against some human cancer cell lines

it in chemotherapeutic aspects. In order to examine the anti-cancer efficacy of any sample against Ehrlich ascites, various techniques are employed including: (1) to determine the amplification in survival time after the utilization of any sample of interest, (2) cytological examination of the ascites cells, after the treatment with the sample of interest, and (3) to quantify the total sum of ascites produced after treatment. In this study, we have employed the last stated way to estimate the effectiveness of hexane fraction.[22]

The effect of hexane fraction was evaluated for *in vivo* efficacy in the EAC model was employed. The main purpose of the existing study was to assess the anti-cancer efficiency of hexane fraction of ethanolic extract of *A. capillus-veneris* aerial part against EAC induced tumor-bearing mice. On visual examinations, toxic signs were not observed externally in animals treated with hexane fraction in terms of their behavioral pattern in respect of their activity and intake of food and water, and also in their general form in respect of their skin and hair texture. The development of EAC is determined by the body weight escalation and increment in the volume of the ascitic fluid. In the present study, the tumor development in EAC induced mice has been observed visually on the basis of amplification of the peritoneal region (Fig. 7).

Body weights of all the animals were calculated frequently on prearranged days. The body weight of the EAC induced mice significantly increased at the end of experiment. The body weight increased to 7 grams in EAC induced mice on day 12, which was more compared to day 1 (Table 1). The increase in body weight might be due to the escalating volumes of ascites by actively proliferating peritoneal EAC cells. In low (50 mg/kg), medium (100 mg/kg), and high (200 mg/kg) dose of hexane fraction treated mice, the increase in body weight was observed to be 4.57, 1.86, and 1.29 grams, respectively, and the values obtained at 100 and 200 mg/kg b.w. were much closer to normal range. While the body weight of positive control mice treated with 5-Fluorouracil (FU) slightly increased (0.57 grams).

Fig. 7: Physical appearance of EAC-tumor bearing mouse and normal mouse

The average volume of ascitic fluid was quantified by sacrificing all the animals at the end of experiment on day 12. Ascitic fluid was collected from each animal from all the experimental groups. Volume collected from the hexane fraction treated animals at dose 200 mg/kg (5.62 ± 1.36) was found to be less as compared to other treatment groups but slightly more than that treated with 5 FU. The tumor cell number in the ascitic fluid of each animal was also calculated after visualizing on haemocytometer. It was observed that the hexane fraction at concentrations 50, 100, and 200 mg/kg body weight treated animals had 68.56×10^7, 48.61×10^7, and 14.80×10^7 tumor cells, respectively, as compared to 82.56×10^7 cells in the saline-treated control group. The positive control group animals received 5-fluorouracil has 5.51×10^7 tumor cells in average. The observations revealed that the hexane fraction reduced the tumor volume and also showed inhibition in tumor cell proliferation.

Finally, this fraction at different concentrations was able to significantly inhibit tumor growth in mice as compared to negative control. At 200 mg/kg i.p. body weight, it caused a superior activity, inhibiting tumor growth at 82.07% while at 50 and 100 mg/kg i.p., caused 16.95 and 41.12%, respectively. Animals of positive control receiving 5-fluorouracil showed 93.32% tumor growth inhibition.

Medicinal plants, phytochemicals, and their analogs were reported to acquire extensive pharmacological activities, including antioxidant, hepatoprotective, anti-inflammatory, anti-cancer, etc. Some of the renowned bioactive compounds, such as, vincristine, vinblastine, podophyllotoxin, camptothecin, taxol, resveratrol, withaferin A, quercetin, and curcumin have been investigated significantly for their capability to treat cancer. Some modified molecules or analogs of such phytochemicals, like topotecan, irinotecan, taxotere, etoposide, and teniposide, etc., have also reported possessing superior anti-cancer properties. There are

Table 1: Effect of hexane fraction on body weight of mice in EAC induced model

			Average body weight (g)			
Groups	*Treatment*	*Dose (mg/kg/i.p.)*	*Day 1*	*Day 5*	*Day 9*	*Day 12*
Group I	Control	0.2 mL n.s.	27.5 ± 1.95	26.3 ± 2.05	30.2 ± 3.85	34.5 ± 3.74
Group II	Hexane fraction	50	27.14 ± 2.03	26.17 ± 1.98	27.57 ± 1.9	31.71 ± 3.35
Group III	Hexane fraction	100	27.28 ± 1.88	26.0 ± 1.91	27.43 ± 3.04	29.14 ± 3.18
Group IV	Hexane fraction	200	27.42 ± 1.71	26.28 ± 1.7	27.57 ± 2.63	28.71 ± 3.25
Group V	5 FU	20	27.43 ± 1.71	26.0 ± 1.82	26.71 ± 1.97	28 ± 2

Table 2: *In vivo* anti-cancer activity of hexane fraction against EAC induced mice

		Day 12					
Treatment	*Dose (mg/kg)*	*Av. volume of ascitic fluid (mL)*	*Av. weight of ascitic fluid (g)*	*Av. no. of tumor cells x 10^7*	*Tumor cell growth (%)*	*Tumor growth inhibition (%)*	*Mortality*
Control	0.2 mL n.s.	10.62 ± 2.08	10.85 ± 2.03	82.56 ± 12.23	100	0	0/10
Hexane fraction	50	9.44 ± 1.72	9.72 ± 1.68	68.56 ± 10.46	83.05	16.95	0/7
Hexane fraction	100	8.74 ± 0.82	9 ± 0.82	48.61 ± 6.52	58.88	41.12	0/7
Hexane fraction	200	5.62 ± 1.36	5.95 ± 1.27	14.8 ± 4.42	17.93	82.07	0/7
5 FU	20	2.17 ± 0.31	2.35 ± 0.3	5.51 ± 1.13	6.68	93.32	0/7

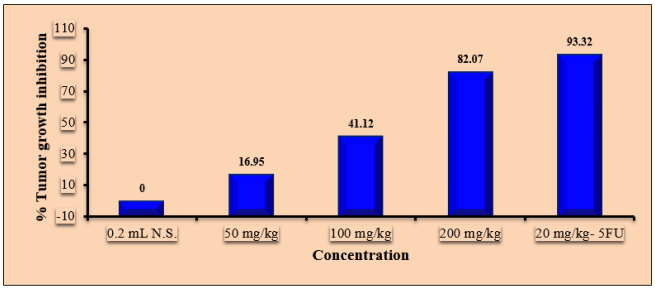

Fig. 8: % tumor growth inhibition of hexane fraction at different concentrations

specifics that these plant secondary products have shown to possess comparatively low side effects.[26] *A. capillus-veneris* has been used in traditional and folklore medicine throughout the world, especially in India, for the treatment of various ailments, and is studied extensively for their pharmacological properties.[27]

However, it is evident (Table 2) that the death rate of EAC cells enhances with an increase in the concentration of the hexane fraction, as depicted in Fig. 8. This fraction was found to be cytotoxic to EAC cells. Hexane fraction was also shown to be non-toxic at all the tested doses, and no mortality was observed.

In the *Unani* system of medicine, *A. capillus-veneris* is used as diuretic, and also used as the best remedy for the treatment of kidney stones from long ago. Its biological activities, like anti-lipolytic, antioxidant, antibacterial,[18] anti-inflammatory, anti-nociceptive,[9,16] and lithotriptic activities[28] clearly rationalize the plant's therapeutic efficacy in nephrolithiasis.

Phytochemical studies of the tested plant extract showed the presence of variety of bioactive compounds, including flavonoids, terpenoids, saponins, tannins, and reducing sugars, etc. All these bioactive compounds have varied biological applications, e.g., antimicrobial, antioxidant, anti-cancer, etc. Phytochemical screenings of medicinal plants play a significant role in recognizing novel sources of industrially and therapeutically essential compounds. A diversity of herbs and herbal products contain different phytochemicals with varied biological activity that can be of valuable therapeutic index.[15,29,30] Many triterpenoidal compounds, such as, isoadiantone, isoadiantol-B, 3-methoxy-4-hydroxyfilicane, and 3,4-dihydroxyfilicane, belonging to adiantane and filicane groups, three flavonoids, such as, quercetin, quercetin-3-O-glucoside, and quercetin-3-O-rutinoside (rutin) were reported to be present in the examined fern. These were identified and isolated via chromatographic techniques. Identities of the separated compounds were established through their physical, chemical, and also through spectroscopic processes, like IR, ^{1}H NMR, 13C NMR, HSQC, HMBC, NOESY, and MS 23.[12]

Our *in vitro* experiment revealed, all the fractions, such as, hexane, chloroform, ethyl acetate, n-butanol, and water of a selected medicinal plant exhibited variety of cytotoxicity against the examined cancer cell lines. Among all, hexane fraction exhibited more antiproliferative effects at 48 hours of treatment against HCT-116, A549, MCF-7, but chloroform fraction proved to be more effective than hexane fraction against MIA PaCa-2 cell line. It is because the bioavailability of herbal remedy varies greatly due to the chemical variation patterns. In actual fact, each fraction of ethanolic extract of *A. capillus-veneris* differs in their quantity and type of bioactive compounds, therefore, resulting in varied bioactivity.[9]

Keeping in view the anti-cancer potential of the fractions of ethanol extract, the present study involves the *in vitro* anti-cancer efficacy of fractions of ethanol extract of *A. capillus-veneris* against four human cancer cell lines. Consequently, the current research was snooping out the *in vivo* anti-cancer potential of hexane fraction in an EAC induced carcinoma in five groups of animals. To investigate the potential of hexane fraction for increased therapeutic benefit against cancer, *in vivo* antitumor activity was performed. The hexane fraction was administered to tumor-bearing mice through an intraperitoneal route, and it noticeably inhibits 16.95 to 82.07% tumor growth in the investigational tumor model of mice, i.e., EAC model of mice.

Conclusion

It could be concluded that all the fractions of ethanolic extract of *A. capillus-veneris* aerial part, revealed to exhibit cytotoxicity towards the tested human cancer cell lines. Hexane fraction, as in respect of low IC_{50} values, was used for *in vivo* anti-cancer studies, where it is proved

to be effective in a concentration-dependent manner by showing the highest tumor growth inhibition in EAC model of mice. The findings of the study indicate that *A. capillus-veneris* herb is an antineoplastic agent and suggests that further studies evaluating the isolation of active antitumor compounds from *A. capillus-veneris* and their mechanism(s) of action are necessary.

Acknowledgements

The authors are grateful to UGC, New Delhi, India, for providing financial support through Moulana Azad National Fellowship. We are also thankful to the Department of Botany, Dr. Harisingh Gour University, and Cancer Pharmacology Laboratory, IIIM-CSIR, Jammu, for providing laboratory facilities and hosting during the course of all this process.

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HOW TO CITE THIS ARTICLE: Ayoub Z, Malik SB, Mehta A. *In vitro* and *in vivo* anti-cancer efficacy of *adiantum capillus-veneris* l. Against some selected human cancer cell lines and on ehrlich ascites carcinoma (EAC) mouse model. Int. J. Pharm. Sci. Drug Res. 2020;12(3):267-274. **DOI:** 10.25004/IJPSDR.2020.120309