International Journal of Advanced Science and Research
ISSN: 2455-4227, Impact Factor: RJIF 5.12
www.allsciencejournal.com
Volume 2; Issue 4; July 2017; Page No. 109-112

A potential effect of *Curcuma aromatica* Salisbury rhizome against Ehrlich ascites carcinoma in Swiss albino mice

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Abstract
The aim of this study was to investigate the potential antitumor effect of the petroleum ether and ethanol extracts of *Curcuma aromatic* rhizome (Zingiberaceae) in Swiss albino mice against Ehrlich Ascites Carcinoma (EAC) cell line. The EAC cells were injected (i.p.) into twenty four mice (divided into 6 numerically equal groups), and after a one-day incubation period, the extracts were administered to the mice daily for 14 days. The antitumor activity of the petroleum ether and ethanol extracts of *Curcuma aromatica* at doses of 200 mg/kg body weight was evaluated against Ehrlich ascites carcinoma (EAC) tumor in mice. The antitumor activity was assessed using mean survival time (MST), tumor volume, tumor cell count, and haematological profile.
Results indicate that the oral administration of the petroleum ether and ethanol extracts of *Curcuma aromatic* increased the mean survival time, tumor volume, tumor weight, body weight and tumor cell count were also significantly reduced. haematological parameters including protein and PCV, which were altered by tumor inoculation, were restored. The effects of both extracts were comparable with standard drug cyclophaspamide.

Keywords: *Curcuma aromatica*, ehrlich ascites carcinoma, tumor volume, viable cell count

Introduction
Malignant ascites is a sign of peritoneal carcinomatosis, the presence of malignant cells in the peritoneal cavity. Tumors causing carcinomatosis are more commonly secondary peritoneal surface malignancies which include: ovarian, colorectal, pancreatic and uterine; extra-abdominal tumors originating from lymphoma, lung and breast; and a small number of unknown primary tumors. (Sangisetty SL *et al.* 2012) [1]. Cancer is the second most common cause of death in the developed world and a similar trend has emerged in the developing countries too. Cancer prevalence in India is estimated to be around 2.5 million, with 8, 00,000 new cases and 5, 50, 000 deaths occurring each year due to this. Cancer is the leading cause of mortality and most of the synthetic chemotherapeutic agents have been reported to exhibit severe normal tissue toxicity, accompanied by undesirable side effects. More over these drugs are highly expensive mutagenic and carcinogenic. Therefore the substitute of the conventional chemotherapeutic agents to control the high mortality rate are needed which will be highly effective at nontoxic doses and inexpensive and accessible to general people. This can be achieved by in-depth research and continuous screening of new molecules or natural agents. Sridharamurthy *et al.* 2012 [2]. Malignant ascites are accounts for approximately 10% of all cases of ascites (Seike M *et al.* 2007) [3].
Herbs have been used as food and for medicinal purposes for centuries. Research interest has been focussed on various herbs that possess antiplatelet, anti-tumor, or immune-stimulating properties that may be useful adjuncts in helping reduce the risk of cardiovascular disease and cancer. Many herbs contain potent antioxidant compounds that provide significant protection against chronic diseases and may also have antiviral or anti-tumor activity. The volatile essential oils of commonly used culinary herbs, spices, and herbal teas inhibit mevalonate synthesis and thereby suppress cholesterol synthesis and tumor growth. (Kumarappan CT et al 2007) [4].
The traditional medicines have played a vital role to treat various diseases in human beings including cancer.
These herbal medications are found in nature and do not have all these ingredients that cause the person to become dependent upon them. *Curcuma aromatica* Salisb wild turmeric is an aromatic and pretty ginger with stout underground rhizomes. The C. *aromatica* Salisb., native to India, Sri Lanka and the Eastern Himalayas.
According to Ayurveda the drug is used in various kinds of diseases related to skin, cardiovascular and respiratory system. Cardiac arrithemia A is used in cosmetic formulations and traditional medicinal applications as an anti-inflammatory agent, to promote blood circulation, to enhance complexion, to remove blood stasis and also for the treatment of cancer (Sikha A et al. 2015) [5].
The *Curcuma Aromatica* contains the active constituents Alpha-curcumene (ar-curcumene), beta curcumene, dcamphor, alpha and beta-turmerone. Also other compounds like d-camphene, p-methoxycinnamic acid, germacrene D, curzerene, germacrone, alpha-andd beta-pinenes, bborneol, alpha-terpeniol, myrcene, terpinolene, gamma-terpinene, limonine, beta-thujone, alpha-copaene, alpha-bergamotene, beta-bisabolene, cuminic aldehyde, cuminyl alcohol, hydroxyisogermafurenolide, xanthorrhizol, curcuphenol, beta –elemene, zingiberene, isoborneol, linalool, beta –farnesene, 1,8-cineole, curzerenone and curcumin. The constituents identified in the oil were: alpha-pinene, beta-pinene, camphene, 1, 8-cineol,isofurano-germacrene, borneol, isoborneol, beta-curcumene, ar-curcumene, xanthorrhizol, germacrone, camphor, and curzerenone.
The chief bioactive constituents are homosesterterpenoids,

curcumin (curcusesterterpene A), (curcusesterterpene B) (curcusesterterpene C). Pharmacological evaluation of *C. aromatica* has been reported on Antioxidant, anti-diabetic, anti-inflammatory, skin problems, antimicrobial, digestive aid, liver protection, arthritis, antibiotic, & wound healing activities. The present study was undertaken to evaluate the effect of petroleum ether and ethanol extracts of *C. aromatica* on EAC bearing ascites cancer in mice cancer (Sikha A *et al.* 2015) [5].

Materials and Methods

The following Instruments and chemical/Drug were used for study : Microscope, Hemocytometer, UV Spectrophotometer, centrifugation apparatus, B. O. D. incubator, Soxlet apparatus, Rotatory evaporator. Drugs and Chemicals- Azoxymethane (AOM) & Dimethylhydrazine, methyl – azoxymethanol & cyclophosphamide. All other reagents and solvents were used analytical A grade.

Collection and Authentication of Plant

The rhizomes of *Curcuma aromatica* were collected from Bhopal, (M.P.) India in the month of December-January 2009. The Rhizomes were authenticated by Prof. P. Patil, Head Department of Botany, Government M.L.B Girls Autonomous College, Bhopal (M.P.) and the voucher specimens no 0999 was deposited in the Departmental Herbarium for future reference.

Preparation of extracts of Curcuma aromatic

The roots of *C. Aromatica* were washed in tap water, cut into small pieces, and then shade dried. The dried pieces were then pulverized with an electric blender, and a yellow powder obtained (25-45 mesh size). The powdered Rhizomes (500 g) was subjected to successive extractions using petroleum ether (40-60) and ethanol in soxhlet apparatus for 72 hours (Kokate CK 1994) [6]. After about forty siphons of each solvent extraction step, the materials were concentrated by evaporation using rotatory evaporator. These extracts were used for further study.

Pharmacological evaluation Animals

Male swiss albino mice (20-25 g) were used for the present study, which were obtained from the Central Animal House, Sagar Institute of Research & Technology Bhopal, M.P. India. Food and water were supplied (*ad libitum*) and kept under controlled temperature 27 ± 2° C with a 12-hour light-dark cycle. The protocol was approved Institutional Animal Ethical Committee under the resolution no SIRT/IAEC/2014/06. The experimental procedures were conducted in accordance to the direction of CPCSEA regulations for the use of laboratory animals,

Acute toxicity studies

The acute oral toxicity study was performed using test guidelines for acute oral toxicity test 425 according to the Organisation for Economic Cooperation and Development. A limit dose of 2000 mg/ kg body weight/oral was used. The signs of toxic effects and/or mortality were observed 4 h after administration, then for the next 48 h. The body weight was recorded for consecutive 14 days (OECD Guideline 2011) [7]. Since the extracts were found safe up to the dose level of 2000 mg/kg body weight, a dose of 200 mg/kg body weight of the ethanol and aqueous extracts was selected for evaluating the effect against Ehrlich Ascites Carcinoma (EAC) in mice.

Induction of Ehrlich ascites carcinoma

Ehrlich Ascites Carcinoma cells were obtained in the peritoneal cavity of mice. They were maintained by weekly intra peritoneal inoculation of 10 cells/mice. Ascites tumor cell counts were done in a Cell Counting machine (Cedex, Roche) using the trypan blue dye exclusion method. Cell viability was > 95%. Tumor cell suspensions were prepared in phosphate balanced salt solution (PBS) at pH 7.4 to final concentrations of 1×10^6 viable cells ml to mice were given intra peritoneal (i.p.) injection of 1×10^6 viable tumour cells per mice in a volume of 0.2 ml (Vogel GH, Vogel WH. 1997) [8, 9]

The animals were divided into four groups (n = 06) and provided with food and water (ad libitum). All animals in each group received EAC cells (2 × 106 cells/mouse ip.) except Group-I: served as normal saline control (5 mL/kg body weight ip.). I Group-II: served as EAC control. Twenty four hours after EAC transplantation, Group-III: animals received EAC control (0.2ml of 2×10^6 cells/mouse), Group-IV: received the standard drug cyclophosphamide (50 mg/kg) (Positive control). The Groups-IV and V, were treated with Petroleum and ethanol extracts of *C. Aromatica* at 200 mg/kg body weight (po.) of respectively for fourteen consecutive days. At 24 h after the last dose was administered, animals were fasted for 18 h, at which point, six animals in each group were sacrificed by cardiac puncture, for estimation of hematological, as well as to measure antitumor activity parameters.

Tumor volume and Packed Cell Volume (PCV)

The mice were dissected for collecting ascitic fluid from the peritoneal cavity. The ascitic fluid of the transplantable tumor was carefully collected with the help of 5 ml sterile syringe. The fluid was subsequently transferred to a graduated glass centrifuge tube and centrifuged at 1000 RPM for 5 min (Sangameswaran B, *et al.* 2012) [10]. The fluid volume was measured. Ehrlich packed cell volume was determined using the following formula

$$\text{Ehrlich Packed Cell volume (\%)} = \frac{\text{1- volume of fluid}}{1} \times 100$$

Median survival time and percentage increase in life span

The tumor growth was monitored by recording the mortality daily for four weeks and percentage increase lifespan (%ILS) was calculated by the following equation.

MST % = Median survival time of treated group–Median survival time of control group/

Median survival time of control group × 100

Median survival time (MST) = (Day of first death + Day of last death)/2

Increase in lifespan =T-C/C × 100 (Sangameswaran B, *et al.* 2012) [10].

Viable and nonviable tumor cell count

The ascetic fluid was collected in a white blood cell (WBC) pipette and diluted 100 times. A drop of the diluted

suspension was then placed on a Neubauer counting chamber and the cells were stained with Trypan blue (0.4% in normal saline). Cells that did not take up the dye were considered viable, while those that did were considered non-viable (Mondal A 2003) [11]. These The viable and nonviable cells were counted using Neubauer chamber (haemocytometer).

Cell count=number of cells × (dilution factor)/
x area × thickness of liquid film

Hematological profile

Collected blood on the 14 day after tumor inoculation was obtained through orbital from mice. The total count blood was drawn into RBC or WBC pipettes, diluted and counted in a neubauer counting chambers. The Sahli's Hemoglobinometer was used for the estimation of hemoglobin concentration (Chandran A, 2003)[12]. Differential count of leukocytes was performed on a freshly drawn blood film using Leishman's stain.

Statistical analysis

All values were expressed as mean ± standard error of mean (S.E.M.). Data were analyzed using one-way ANOVA followed by a Dunnett multiple comparison test $p \leq 0.01$ was considered as statistically significant.

Results and Discussion

The present study was to investigate the anti-tumor activity of the petroleum ether and ethnolic extracts of *C. Aromatica* in EAC tumor infected mice.

No toxic effects were observed after treatment with rhizome extracts of *Curcuma aromatic* at higher dose of 2000 mg/kg body weight. Hence, there were no lethal effects. The dose were chosen from each extracts 200mg/kg. for further experimentation

The best parameters for gauging the effectiveness of any anticancer agents are the prolongation of mean survival time and life span of experimental animals (Sridharamurthy NB, 2011) [2]. In the present study the median survival time and percentage life span of mice increased in the extracts and cyclophaspamide treated group. The life span increased in standard 61.89% petroleum ether 53.85% and ethanolic 68.42% compared with control group. Table -1

Ascitic tumor implantation promotes local inflammatory reactions leading to increase in vascular permeability, and results in intense edema formation, cellular migration and progressive ascitic fluid formation. Ascitic fluid is essential to tumor growth, since it constitutes the direct nutritional source for tumor cells (Chatterjee D 2011) [13]. The reduction of tumor volume, viable and nonviable cell count finally reduces the burden and amplified the life span of EAC bearing mice. In our study a regular rapid increase in ascetic fluid tumor volume, packed volume and viable cell was observed in EAC control tumor bearing mice. Treatment with petroleum ether and ethnolic extracts 200mg/kg Significantly suppressed the tumor volume and packed volume count of the tumor bearing mice. Significantly decrease in viable cell count and increase in non-viable cell count in tumor bearing mice indicate the antitumor activity against EAC cells in mice. Ethanolic extract shows highly effective as compared to petroleum ether extracts. The results indicate that the petroleum ether and ethanolic extracts reduce the nutritional fluid volume and inhibiting the tumor growth increases the life span of EAC-bearing mice. Table -2

The major problems in the cancer chemotherapy are myelosupression and anemia (Panesar K, 2013) [14] Mainly the reduction of RBC and Hb% in tumor bearing mice lead to the formation anemic condition and this may occur either due to iron deficiency or due to hemolytic or myelopathic conditions (Sridharamurthy NB, 2011) [2]. In the present study the Hb content increase compared with the control group. Treatments with both extracts treated group and cyclophaspamide group showed significant improvement in Hb content RBC level, WBC and differential count compared with control group. The ethanolic extract treated group showed highly significant improvement hematology profile in tumor beraring mice. The results shown in table-3

These findings clearly indicate that the rhizome extracts of *Curcuma Aromatica* possess effective qualities aginst EAC tumor bearing mice, which might be due to presence of bioactive constituents such as carbohydrates, alkaloids, glycosides, phenolic compound, homosesterterpenoids, and curcumin. (curcusesterterpene A), (curcusesterterpene B) (curcusesterterpene C). The curcumin are chief active constituents which play a vital role in the treatment of cancer. The curcumin effects on the activation of apoptotic and anti-angiogenic pathways in Ehrlich Ascites Tumor (EAT) cells. It inhibits the proliferation of EAT cells and ascites formation. The induction of apoptosis in EAT cells showed nuclear condensation, DNA fragmentation and translocation of caspase-activated DNase (CAD) to nucleus. Curcumin-induced apoptosis is mediated through activation of caspase-3, which is specifically inhibited by the caspase-3 inhibitor, Ac-DEVD-CHO. On the other hand, the decreased secretion of ascites by EAT cells is corroborated by reduction in VEGF secretion upon curcumin treatment. Curcumin inhibits the proliferation of endothelial cells in mouse peritoneum and furthermore, inhibitis of nuclear translocation of NF-kB p65, a transcription factor required for VEGF gene expression, in EAT cells.

Further *invivo* studies of these bioactive constituents are deemed necessary in order to develop curcumin-based anticancer strategies. In view of the increasing interest in the association between curcumin and cancer initiation and progression, this important field is likely to witness expanded effort and to attract and stimulate further vigorous investigations

Conclusion

The rhizome extracts of *Curcuma aromarica* exhibited antitumor activities against Ehrlich Ascites Carcinoma in Swiss albino mice. Animals induced with EAC cell lines (at a dose of 2 106 cells) and then after 24hrs, treatment with ethyl acetate and alcohol extract at a dose of 100mg and 200mg/kg, for a period of 14 days. Later the blood samples were collected and animals were sacrificed for studying of antitumor activity. Treatment with petroleum ether and ethanolic extracts 200 showed significant increase in the survival time and life span in tumor bearing rats. The reduction of tumor volume, packed cell volume and viable cell count were observed in both petroleum and ethanolic extract when compared with fluorouracil treated and EAC control tumor bearing mice. Hematological parameters were altered in EAC treated animals, were restored considerably in

both extracts treated animals, However ethnolic extracts showed higly potent as compared to petroleum ether extracts. The results of all the parameters, clearly indicates that the overall effects of plant *Curcuma aromatic* is mainly due curcumin it contains. The inhibitory property of the extracts of *Curcuma aromatic* on EAC cell lines can be considered further studies in the treatment of cancer.

Table 1: Effect of *C. Aromatica* on Median Survival Time, Percentage increase of Life Span in tumor bearing mice

Treatment Groups	Median Survival Time (Days)	Increase of Life Spain (%)
Control	22.32±0.37	-
Standard	34.50±0.53**	61.89±0.47**
Pet. ether extract 200mg/kg. b.wt	36.95±0.21*	13.85±1.39*
Ethanolic extract 200mg/kg b.wt	32.23±0.45*	64±.0.47**

The values are expressed as mean ± SEM. (n=6) Days of treatment = 14; *P < 0.001 as compared to control group.

Table 2: Effect of *C. Aromatica* on Tumor Volume, Packed cell volume, Viable and Nonviable cell count in tumor bearing mice

Treatment Groups	Tumor Volume	Packed cell volume (ml)	Viable cells 1×10^6 cells/ml	Nonviable cells 1×10^6 cells/ml
Control	3.25±0.10	3.11±0.25	13.83±1.12	0.08±0.018
Standard	0.95±0.005	1.89±0.11***	2.24±0.97***	2.43±0.014**
Pet. ether extract 200mg/kg b.wt	2.53±0.08	1.75±0.12*	7.85±1.01*	1.18±0.011*
Ethanolic extract 200mg/kg b.wt	1.86± 0.06	2.10±0.16**	6.84±1.12**	1.04±0.024*

The values are expressed as mean ± SEM. (n=6) Days of treatment = 14; *P < 0.001 as compared to control group

Table 3: Effect of *C. aromatica Salisbury* on hematological Profile in ECA tumor bearing mice

Treatment Groups	Hb (gm%)	RBC (million/mm^3)	WBC (10^3 cells/mm^3)	Differential Count (%)		
				Lymphocytes	Neutrophils	Eosinophils
Control	7.48±0.64	2.19±0.22	17.78±1.24	35.54±1.34	53.59±1.42	12.59±1.93
tandard	11.07±0.17	3.91±0.13*	8.56±0.83*	54.65±1.71*	36.16±1.23*	769±1.16*
Pet. ether extract 200mg/kg b.wt.	10.35±0.23	3.68±0.27	10.11±0.79	49.13±0.37*	41.54±0.18*	7.33±0.27
Ethanolic extract 200mg/kg b.wt.	11.85±0.31	4.87±0.14*	8.35±0.33**	53.66±0.24*	40.17±0.21*	5.17±0.11*

Values are mean S.E.M. where n=6 *p< 0.001statistically significant when compared with normal group. **p < 0.001statistically significant when compared with EAC control group.

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