*Research Article*

Available Online at: www.ijpir.com

**International Journal of Pharmacy and Industrial Research**

ISSN Print 2231 – 3648
Online 2231 – 3656

# Evaluation of the antitumour effect of Cynodon dactylon and Aristolochia bracteata against Ehrlich ascites carcinoma (EAC) induced mice

**Abdul Mudasir Mohammed[1], Abdul Razzaq Mohammed[2]**

[1]Assistant Professor, Vijay College of Pharmacy, Nizamabad, Telangana
[2]Researcher, Governors State University, University Park, IL, USA

## ABSTRACT

### Aim

Evaluation of the antitumour effect of Cynodon dactylon and Aristolochia bracteata against Ehrlich ascites carcinoma (EAC) induced mice.

### Methods

Antitumor activity of chloroform extract with various doses of Cynodon dactylon and chloroform extract of Aristolochia bracteata was evaluated against Ehrlich ascites carcinoma (EAC) tumor mice. Acute and short-term toxicity studies were performed initially in order to ascertain the safety of chloroform extract of Cynodon dactylon and Aristolochia bracteata. After 24 h of tumor inoculation, the extract was administered daily for 14 days. After the administration of the last dose animals (mice) were made to fast for 18 h then sacrificed for evaluation of antitumor activity. The effect of Chloroform extract of Cynodon dactylon and Aristolochia bracteata on the growth of transplantable liquid tumor, life span of EAC bearing Swiss Albino mice and simultaneous alterations in hematological profile were estimated.

### Results

The Chloroform extract of Cynodon dactylon and Aristolochia bracteata showed significant ($P<0.05$) decrease in tumor volume, packed cell volume and viable cell count, and increased the nonviable cell count and mean survival time thereby increasing life span of EAC tumor bearing mice. Haematological profile reverted to more or less normal levels in extract treated mice.

### Conclusion

The Chloroform extract of Cynodon dactylon and Aristolochia bracteata exhibited statistically significant antitumor effect depending on dose and duration of treatment.

**Keywords:** Cynodon dactylon, Aristolochia bracteata, Ehrlich ascites carcinoma, Mean survival time, Viable cell count.

---

**Author for Correspondence:**
Abdul Mudasir Mohammed
Assistant Professor,
Vijay College of Pharmacy,
Nizamabad, Telangana

## INTRODUCTION

Cancer continues to represent the largest cause of mortality in the world and claims over six million lives every year. [1] An extremely promising strategy for cancer prevention today is chemo prevention, which is defined as the use of synthetic or natural agents (alone or combination) to block the development of cancer in humans. Plants such as herbs and shrubs are used in traditional medicine have been accepted currently as one of the main source of cancer chemoprevention drug discovery and development. [2] There is overwhelming demand for the pharmacological evaluation of plants used in Indian traditional system of medicine. Plant derived natural products such as alkaloids, flavonoids, resins, tannins, have received tremendous attention in the sight of researchers due to their diverse pharmacological properties including antioxidant and antitumor activity [3, 4]. Antioxidants which are obtained from the plants are inhibiting the scavenging radicals, by ultimately providing protection to humans against degenerative diseases.

Epidemiological studies have suggested that certain dietary compounds are associated with lowering of cancer risk. [5] These dietary supplements include vitamins as well as other phytochemicals, particularly polyphenols. Because most of phytochemicals are safe at the levels they found in the diet the plants like Actinidia macrosperma, popularly called cat ginseng is useful in the treatment of cancer. [6] The polyphenolic present in most plants shown in vitro antioxidant activity and inhibits the growth of cancer cell culture. [7, 8, 9] Anticancer activity of Scutellaria baicalensis shown potent activity against human malignant brain tumor cells. [10] Recruitment of new blood vessels or increasing level of cytotoxic T-lymphocytes may be a possible role of some of phytochemicals, example Convolvulus arvensis. [11] Also Indigofera aspalathoides shown potent antitumor activity against EAC induced mice and several phytochemicals containing plants shown a promising anticancerous agents that are non toxic to normal cells. CECD phytochemical investigation shows the presence of alkaloids, tannins, flavonoids and earlier research activities shows potent antioxidant, [12] cardioprotective, [13] and antiulcer activity. [14] CEAB scientifically validated for antioxidant, [20] anti-inflammatory activity [19] and also used in liver disorders. On all these basis the present study of evaluation of anticancer activity of CECD and CEAB was carried out in EAC induced mice.

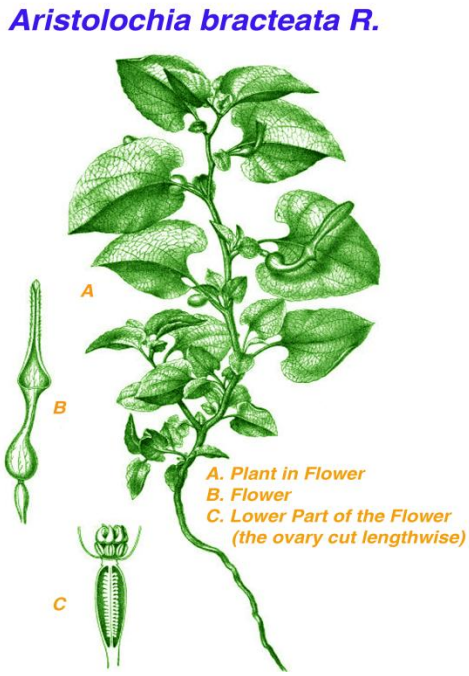
## PLANT PROFILE

India, with its varied climatic conditions, is a repository of a rich and diverse flora, which is the mainstay of well-organized Indian traditional systems of medicine viz. Ayurveda and Siddha. Plants belonging to family Poaceae, which is widely distributed throughout India and frequently used in traditional medicines.

***Cynodon dactylon***

India : Doob
English : Dhub grass, Bermuda grass, Bahama grass

*Aristolochia bracteata Retz* (Family: Aristolochiaceae)




### Synonyms

A.abyssinica klotzsch, A.bractealata Lam, A.lotschyi, A.rich.

### Vernacular names of plant

Sanskrith : Dhumrapatra [15], Pattra-bhanga [16]
English : Bracteated birthwort [17, 18]

## MATERIAL AND METHODS

### Preparation of plant extract

The whole plant was authenticated by the Botanist of Srivenkateshwara University- Dr. Madhava Chetty. It was collected in the ideal conditions, washed with 2% $KMnO_4$ then air dried under shade and powdered to a fine and uniform texture by passing through sieve no. The powder was extracted by successive solvent extraction method using petroleum ether, chloroform and methanol for 24 hrs/cycle. The extract obtained was concentrated and reconstituted with saline & administered orally daily in single dose.

### Animals

Female Swiss Albino mice weighing between 20 –30 g were used for the study. Food and water were supplied (ad libitum) and the animals were kept in a 12 h light: 12 h dark cycle and environment temperature (23 ± 1 $^0$C) in standard propylene cages. All the animals were monitored till the end of the study and sacrificed for weighing the organs and finally the dead animals were incinerated and discarded. All experiments were conducted in accordance with the direction of Institutional Animal Ethics Committee, Deccan School of Pharmacy, Hyderabad.

### Treatment groups

The animals were divided into eleven groups of 12 animals and to the animals from group – 2 to 11 one million cancer cells were transplanted i.p. The animals are grouped as follows:

Group-1: serve as normal without any administration
Group-2: serve as tumor control, without any treatment
Group-3: serve as standard (Cyclophosphamide 5 mg/kg)
Group-4: chloroform extract of C.dactylon administered (50 mg/kg)
Group-5: chloroform extract of C.dactylon administered (100 mg/kg)

Group-6: chloroform extract of C.dactylon administered (200 mg/kg)
Group-7: chloroform extract of C.dactylon administered (250 mg/kg)
Group-8: chloroform extract of A.bracteata administered (50 mg/kg)
Group-9: chloroform extract of A.bracteata administered (100 mg/kg)
Group-10: chloroform extract of A.bracteata administered (200 mg/kg)
Group-11: chloroform extract of A.bracteata administered (250 mg/kg)

### Induction of Ehrlich Ascites Carcinoma

Mice (donor) bearing the ascitic carcinoma was taken 15 days after tumor transplantation. A sterile syringe with 18 guage was used to draw the ascitic fluid from the body of mice. A small amount of ascitic fluid was tested for microbial contamination. Tumor viability was determined by Trypan blue exclusion test and cells were counted by using haemocytometer. The ascitic fluid was suitably diluted in normal saline to get a concentration of $10^6$ cells /ml. of tumor cell suspension. This was injected intraperitonially to obtain ascitic tumor. Weight of the mice was recorded on the day of tumour inoculation and after that every day for 3 days. Treatment was started 24 hours after tumor inoculation. Cyclophosphamide was orally administered daily to the standard group. Extracts of different doses were administered to the animals from group – 4 to 11 till the $14^{th}$ day orally. [20]

Evaluation parameters:

1. Percentage increase in body weight as compared to day- 0.
2. Mean survival time (MST).
3. Percentage increase in life span (% ILS).
4. Percentage increase in life span (% ILS).
5. Cell viability test (% survivors of malignant cells in ascitic fluid). [21-25]
6. Hematological parameters: a) Total W.B.C. and DLC b) Total R.B.C. and Hemoglobin content.

## RESULTS

**Table-1.Effect of drugs on body weight changes in tumor induced mice**

| Group | Day-0 | Day-3 | Day-6 | Day-9 | Day-12 | Day-15 |
|---|---|---|---|---|---|---|
| Control | 23.62± 0.72 | 24.420± 0.73 | 25± 0.76 | 27.9± 1.14 | 27.57± 1.17 | 27.25± 1.51 |
| Cyclopho sphamide 5 mg/kg | 23.77± 0.54 | 24.3± 0.48 | 25.68± 0.43 | 25.42± 0.46 | 25.99± 1.03 | 26.99± 0.83 |
| CECD | | | | | | |
| 50 mg/kg | 24.83± 0.31 | 25.08± 0.16 | 24.37± 0.21 | 24.53± 0.14 | 24.58± 0.33 | 25.05± 0.23 |
| 100 mg/kg | 24.30± 0.85 | 24.48± 0.94 | 24.03± 0.91 | 23.93± 1.005 | 23.78± 1.34 | 24.28± 1.28 |
| 200 mg/kg | 25.25± 0.46 | 25.52± 0.50 | 25.98± 0.35* | 26.0± 0.41* | 26.0± 0.34* | 26.28± 0.33 |
| 250 mg/kg | 27.33± 0.61 | 28.20± 0.66 | 27.1± 0.60 | 25.43± 1.19 | 25.13± 0.91* | 25.23± 0.84 |
| CEAB | | | | | | |
| 50 mg/kg | 24.83± 0.33 | 26.72± 0.44** | 25.80± 0.86 | 25.13± 0.93** | 24.28± 1.24 | 23.67± 1.62 |
| 100 mg/kg | 23.67± 0.82 | 24.03± 0.80 | 23.9± 1.15 | 23.47± 1.10 | 24.77± 0.88 | 23.43± 1.47 |
| 200 mg/kg | 27.25± 0.36 | 26.92± 0.24 | 26.77± 0.47 | 26.17± 0.53 | 25.43± 0.59 | 25.18± 0.77 |

| | | | | | | |
|---|---|---|---|---|---|---|
| 250 mg/kg | 25.83± 0.307 | 25.8± 0.553 | 26.6± 0.509 | 24.83± 0.302∗ | 24.35± 0.453 | 23.97± 0.605 |

Different groups of Female Swiss Albino mice weighing between 20-30 gms were taken. To each group of animal's equal proportion of cancer cells were administered. After 24 hrs of inoculation different doses of CECD and CEAB were administered to different groups and their weights were constantly seen for every 3 days. Data collected as mean ± SEM and analysed by student-t test by comparing control group with normal mice and extracts treated are compared with negative control group. P<0.05 is significant. n=6. ∗P<0.05, ∗∗P<0.01.

**Table-2.Effect of different concentrations of drugs on Blood cells (WBC, RBC, PLT, LYM) in tumor induced mice**

| Group | WBC ($x10^3/\mu L$) | RBC ($x10^6/\mu L$) | PLT ($x10^3/\mu L$) | LYM % |
|---|---|---|---|---|
| Normal | 8.165± 0.6781 | 7.845± 0.2761 | 840.2± 61.49 | 88.82± 1.681 |
| Negative Control | 16.03± 0.5909∗∗ | 7.472± 0.1799 | 571.2± 54.94∗ | 84.32± 5.575 |
| Cyclophos-phamide | 9.267± 0.7338∗∗ | 7.883± 0.1752 | 1251± 122.0∗∗ | 86.05± 4.470 |
| CECD | | | | |
| 50 mg/kg | 9.517± 0.4729∗∗ | 6.90± 0.3682 | 690.3± 65.22 | 93.48± 0.9810 |
| 100 mg/kg | 10.69± 0.85∗∗ | 6.88± 0.30 | 772.3± 69.85∗ | 89.23± 1.933 |
| 200 mg/kg | 12.5± 0.4137∗ | 8.20± 0.1787∗ | 713.3± 62.82 | 92.07± 0.9587 |
| 250 mg/kg | 12.23± 0.7762∗ | 7.00± 0.4499∗ | 626.2± 77.14 | 90.95± 1.119 |
| CEAB | | | | |
| 50 mg/kg | 10.68± 0.8382∗∗ | 7.269± 0.4937 | 850.8± 103.9 | 86.05± 4.470 |
| 100 mg/kg | 9.677± 1.404∗∗ | 7.30± 0.5256 | 885.2± 93.97∗ | 88.17± 3.660 |
| 200 mg/kg | 5.877± 0.7614∗∗ | 7.456± 0.6244∗ | 814.3± 69.64 | 89.90± 2.163 |
| 250 mg/kg | 5.70± 0.8869∗∗ | 5.932± 0.6497∗ | 551± 106.8 | 87.3± 2.157 |

Different groups of Female S.Albino mice weighing between 20-30 gms were taken. To each group of animal's equal proportion of cancer cells were administered. After 24 hrs of inoculation different doses of CECD & CEAB were administered to different groups. On $15^{th}$ day the blood is drawn retro-orbitally and blood cells such as WBC, RBC, Platelets, and Lymphocytes were counted by the digital cell counter with a model number Sysmex KX [30]. Data collected as mean ± SEM and analysed by student t test by comparing control group with normal mice and extracts treated are compared with negative control group. P <0.05 is significant. n=6. ∗P<0.05, ∗∗P<0.01

**Table-3.Effect of different concentrations of drugs on blood cells in tumor induced mice**

| Group | HGB g/dL | PCV % | MCV fL | MCH Pg | MCHC /dL |
|---|---|---|---|---|---|
| Normal | 11.80± | 36.23± | 49.10± | 16.00± | 32.58± |
| | 0.4858 | 1.247 | 0.6899 | 0.5013 | 0.839 |
| Negative-control | 12.73± | 39.10± | 48.95± | 15.95± | 32.63± |
| | 0.3180 | 1.296 | 0.9615 | 0.1384 | 0.4544 |
| Cyclophos-phamide | 11.90± | 36.65± | 48.35± | 15.83± | 33.17± |
| | 0.5477* | 1.656 | 0.6520 | 0.1726 | 0.5270 |
| CECD | | | | | |
| 50 mg/kg | 10.63± | 33.52± | 49.3± | 15.63± | 31.73± |
| | 0.643** | 2.009* | 0.77 | 0.24 | 0.335 |
| 100 mg/kg | 10.5± | 33.9± | 49.3± | 15.3± | 31.0± |
| | 0.5*** | 1.67* | 0.67 | 0.259* | 0.414*** |
| 200 mg/kg | 12.23± | 41.25± | 50.2± | 14.9± | 29.68± |
| | 0.304*** | 1.126 | 0.66 | 0.360*** | 0.473*** |
| 250 mg/kg | 9.150± | 28.85± | 47.82± | 15.20± | 31.88± |
| | 0.73** | 2.669** | 0.9046 | 0.1713** | 0.4143 |
| CEAB | | | | | |
| 50 mg/kg | 11.90± | 36.65± | 48.35± | 15.83± | 33.17± |
| | 0.547 | 1.656 | 0.6520 | 0.1726 | 0.5270 |
| 100 mg/kg | 11.07±0.9054 | 32.08± | 46.83± | 16.38± | 35.12± |
| | | 3.241* | 1.107 | 0.5924 | 1.963 |
| 200 mg/kg | 11.98± | 36.27± | 48.35± | 16.13± | 33.43± |
| | 0.9199 | 3.461 | 0.6908 | 0.1909 | 0.8325 |
| 250 mg/kg | 9.5± | 30.16± | 48.24± | 16.34± | 33.84± |
| | 1.349* | 2.814* | 0.8041 | 0.4190 | 0.5250 |

Different groups of Female S.Albino mice weighing between 20-30 gms were taken. To each group of animal's equal proportion of cancer cells were administered. After 24 hrs of inoculation different doses of CECD & CEAB were administered to different groups. On 15$^{th}$ day the blood is drawn from retroorbital plexus and various hematological parameters such as Hb, PCV, MCV, MCH & MCHC were measured by the digital cell counter with a model number- Sysmex KX [30]. Data collected as mean ± SEM and analysed by student-t test by comparing control group with normal mice and extracts treated are compared with negative control group. $P<0.05$ is significant. n=6. *P<0.05, **P<0.01, ***P<0.001

**Table-4. Effect of different concentrations of drugs on peritoneal cells (million/ml) in tumor induced mice**

| Group | Mean |
|---|---|
| Negative control | 39±0.64 |
| Cyclophosphamide (5 mg/kg) | 13.80±1.25* |
| CECD | |
| 50 mg/kg | 2.6±0.12* |
| 100 mg/kg | 7.17±0.23* |
| 200 mg/kg | 4.74±0.384* |
| 250 mg/kg | 16.1±0.57* |

| | |
|---|---|
| CEAB | |
| 50 mg/kg | 51.53±0.72∗ |
| 100 mg/kg | 22.23±0.593∗ |
| 200 mg/kg | 17.33±0.338∗ |
| 250 mg/kg | 15.17±0.12∗ |

Different groups of Female Swiss Albino mice weighing between 20-30 gms were taken. To each group of animal's equal proportion of cancer cells were administered. After 24 hrs of inoculation different doses of chloroform extract of A, bracteata and C. Dactylon were administered to different groups. On 15th day the peritoneal fluid is withdrawn from the peritoneal cavity and number of viable cells are counted on Neobaurs chamber. Data collected as mean ± SEM and analyzed by student t test by comparing control group with normal mice and extracts treated are compared with negative control group. P <0.05 is significant. n=6. ∗P<0.01

**Table-5.Effect of drugs on Spleen and Thymus weight in tumor induced mice**

| Group | Wt of spleen (gms) | Wt of Thymus (gms) |
|---|---|---|
| Normal | 0.313±0.003 | 0.07±0.005 |
| Negative control | 0.21±0.033∗∗ | 0.053±0.006∗∗ |
| Cyclophosphamide | 0.16±0.015 | 0.07±0.007 |
| CECD | | |
| 50 mg/kg | 0.192±0.005∗ | 0.097±0.003∗∗ |
| 100 mg/kg | 0.25±0.007 | 0.13±0.004∗∗ |
| 200 mg/kg | 0.213±0.007 | 0.07±0.017 |
| 250 mg/kg | 0.173±0.01 | 0.102±0.014∗ |
| CEAB | | |
| 50 mg/kg | 0.17±0.011 | 0.072±0.002∗ |
| 100 mg/kg | 0.265±0.009 | 0.077±0.004∗ |
| 200 mg/kg | 0.147±0.008 | 0.083±0.006∗ |
| 250 mg/kg | 0.21±0.027 | 0.072±0.005∗ |

Different groups of Female Swiss Albino mice weighing between 20-30 gms were taken. To each group of animal's equal proportion of cancer cells were administered. After 24 hrs of inoculation different doses of chloroform extract of A,bracteata and C.dactylon were administered to different groups. On 15th day the animals were sacrificed, their spleen and thymus gland is removed and weighed. Data collected as mean ± SEM and analysed by student t test by comparing control group with normal mice and extracts treated are compared with negative control group. P <0.05 is significant. n=6.∗P<0.05, ∗∗P<0.01

**Table-6.Effect of different concentration of extracts on tumor living cell number in Ascitic fluid of tumor induced mice**

| Drug | Mean % Death After 3 hrs | | | |
|---|---|---|---|---|
| | 10 µg / ml | 20 µg / ml | 40 µg / ml | 50 µg / ml |
| Cyclophosphamide | 15.3 | 100 | 100 | 100 |
| CEAB | 19.8 | 68.3 | 61.1 | 68 |
| CECD | 74 | 82.3 | 91 | 96 |

Control = 6 %

The ascitic fluid was withdrawn from animals which were induced with EAC cell lines, 0.1 ml of ascitic fluid was aspirated, and it was diluted with 2ml of phosphate buffered saline (PBS). To this equal volume of different extracts were mixed and this mixture was incubated for 3 hrs at 37° C. Then the mixture was added to 0.5ml of tryphan blue and mixed thoroughly. The diluted suspension was charged into hemocytometer. The viable cells were (unstained) counted in a WBC chamber under a microscope and the mean numbers of cells in four chambers were calculated and expressed in terms of mean percent death.

## DISCUSSION

The great majority of chemical identified as cytotoxic to cancer cells are generally also toxic to normal cell. Nevertheless, the potentiation of host defense mechanisms has been recognized as a possible means of inhibiting tumor growth without harming the host. Therefore, searching for immune enhancement effects may have great potential in cancer treatment. Our immune system is one of the body vital defenses to fight against cancer. When normal cells transform into cancerous cells, few of the antigens on their surface change. New or altered antigens alerts the immune defenders, which includes cytotoxic T cells, natural killer cells, and macrophages. Tumors develop when the monitoring system breaks down or is overwhelmed. Some tumors may escape from the immune defenses by hiding or disguising their tumor antigens. On the other hand, tumors may survive by encouraging the production of suppressor T cells; these T cells act as the tumor's allies, blocking cytotoxic T cells that would normally attack it. On the basis of this, more versatile plants used as a source of active phytochemicals from CECD and CEAB. In the present studies, the hematological parameters including hemoglobin content and RBC count was increased significantly and along with lymphocytes count and platelet count dose dependent manner. The relation between immune states and the occurrence, growth and decline of tumor is one of the essential problem in tumor treatment. Earlier report suggest that various biological response modifiers which are natural products having biological activity to enhance host defense system have been considered as useful to inhibit tumor growth in cancer immunology. [26, 27] In the previous studies carried out in our laboratory it has been proved that, both of these plant extracts possess antiallergic and antiarthritic activities. [28] These studies supported the earlier possible mechanism of TNF-α and other cytokines depletion. [29] Based on the results of present study and previous studies, the effect of these plant extracts life prolonging action may be due to the above said mechanism.

The hematological data in our study shown an increased level of leucocytes, and other health parameters, including Hb, MCV, PCV, MCH, and MCHC indicating strengthening non-specific immunity. In nonspecific immunity, macrophages play an important role in host defense mechanism [29] through producing TNF-α and nitric oxide. Based on these results, we report that, chloroform extract of C.dactylon and A. bracteata extracts dose dependently improved non specific immune functions in EAC induced tumor mice thereby enhancing lymphocytes proliferations, NK cell cytotoxicity, macrophage fraction and secretion of antibody and rise in effectors molecule TNF-α, and leading to inhibition of carcinogenesis and metastasis and prolonging total survival time. The plant extracts produced insignificant gain in spleen and thymus gland weight in tumor induced mice. These results indicate that, the anticancer activity does not involve activation and regulation of hemopoietic system. In both the extracts treated animals showed significant increased mean percentage death of tumor cells indicates direct and indirect cytotoxicity on tumor cells. The anticancer activity shown by both the plant extracts may be due to the presence of the most important active constituents like alkaloids, polyphenolic and flavonoids.

## CONCLUSION

We summarize and conclude from the present study that the *Aristolochia bracteata* and *Cynodon dactylon* chloroform extract possess anti-tumor activity against EAC induced in mice. Based on the previous reports and present results, probably the active constituents present in the plant preparations including alkaloids, flavonoids and polyphenolics produced immunomodulator activity

by increasing non-specific immune cells activation and numbers. They may also produced the present activity by regulating the level of cytokines, including TNF, IL, IFN, LK, NO, and free radicals at cellular level. The decrease in total body weight, tumor size and increased total survival time reflects the efficacy and safety of therapy. These results are also supported by their higher therapeutic index as shown in acute toxicity study. Hence, we conclude that the treatment with these plant preparations could be of high efficacious and safer than compared to existing medication. Hereby, we conclude that the extracts of *A.bracteata and C.dactylon* possess effective and safe anticancer activities. However, more detailed studies are required to establish molecular mechanism of action of these preparations.

## REFERENCES

[1]. Abdullaev FI, Luna RR, Roitenburd BV, Espinosa AJ. Pattern of childhood cancer mortality in Mexico. Arch Med Res 31, 2000, 526-31.
[2]. Abdullaev FI. Plant derived agents against cancer. *In*: Gupta SK, editor. Pharmacology and therapeutics in the new millennium. Narosa publishing House: New Delhi, India; 2001, 345-54.
[3]. I.C.A.R. Bull, NO 25, Appx. 1(3), 1946.
[4]. Nadkarni KM, Nadkarni AK. Indian Materia Medica. Mumbai: Popular Prakashan 1996.
[5]. Gerber M. The comprehensive approach to diet: a critical review. Nutrition 131, 2001, 3051-55.
[6]. Yin L, Fan J, Zhao Y et al. Immunomodulatory activity of aqueous extract of *Actidinia macrosperma*. Asia Pac J Clin Nutr 16, 2007, 261-65.
[7]. Yamaguchi F, Yoshimura Y, Nakazawa H, Ariga T. Free radical scavenging activity of grape seed extract and antioxidants by electron spin resonance spectrometry in an $H_2O_2$/NaOH/DMSO system. J Agric Food Chem. 47, 1999, 2544-48.
[8]. Agarwal C, Sharma Y, Zhao J, Agarwal R. Apolphenolic fraction from grape seeds causes irreversible growth inhibition of breast carcinoma MDA-MB468 cells by inhibiting mitogen-activated protein kinases activation and inducing G1 arrest and differentiation. Clin, Cancer Res. 6, 2000, 2921-30.
[9]. Sharma G, Tyagi AK, Singh RP, Chan DC, Agarwal R. Synergistic anticancer aeffects of grape seed extract and conventional cytotoxic agent doxorubicin against human breast carcinoma cells. Breast cancer Res Treat. 85, 2004, 1-12.
[10]. Scheck AC, Perry K, Hank NC, Clark WD. Anticancer activity of extracts derived from the mature roots of Scutellaria baicalensis on human malignant brain tumor cells. BMC Complementary and Alternative Medicine. 16, 2006, 1-9.
[11]. Riordan NH, Meng X, Riordan HD. Anti-angiogenic, Anti-tumor and Immunostimulatory Effects of a Non Toxic Plant Extract. Presented at Comprehensive Cancer Care, Arlington, Virginia, 2000.
[12]. Auddy B, Ferreira M, Blasina F, Lafon L, Dajas F, Tripathi PC, et al. Screening of antioxidant activity of three Indian medicinal plants, traditionally used for the management of neurodegenerative diseases. J Ethnopharmacol 84(2-3), 2003, 131-38.
[13]. Najafi M, Nazemiyeh H, Garjani A, Ghavimi H, Gharekhani A. Cardioprotective effects of *Cynodon dactylon* against ischemia/reperfusion induced arrhythmias. J Mol Cell Cardio 2007. Available from URL://www.sciencedirect.com/scienceob =article.
[14]. Patil MB, Jalapure SS, Prakash NS, Kokate CK. Antiulcer properties of alcoholic extract of *Cynodon dactylon* in rats. Trad Med Neutra. 2005. Available from: URL:http://octahort.org/books/680/680_16html.
[15]. Council of Scientific and Industrial research. Revised ed. The wealth of India: Raw Materials; New Delhi, India 2003, 422-27.
[16]. Nadkarni KM. Indian Materia Medica. Mumbai. Popular Prakashan Pvt Ltd; 1998, 138-39.
[17]. Sastry JL, Dravyaguna Vijana. 2nd ed. Varanasi: Chowkhamba orientalia, 2, 2005, 912-13.
[18]. Pandey G, Dravyaguna Vijana. Varanasi: Chowkhamba Krishnadas Academic 2, 2004, 266-67.
[19]. Shirwaiker A, Somashekhar AP. Anti-inflammatory activity and free radical scavenging studies of *Aristolochia bracteata* Lam. Indian journal of Pharma Sci 65(1), 2003, 67-9.

[20]. Uma Devi P, Rao BSS and Solomon FE "Effect of plumbagin on the radiation induced cytogenetic and cell cycle changes in mouse Ehrlich ascites carcinoma *in vivo"*. Ind J Exp Biol 36, 1998, 891-95.
[21]. Echardt AE, Malone BN and Goldstein I Cancer Res 42, 1982, 2977.
[22]. Umadevi P, Emerson Soloman F, and Sharada AC Indian J Exp Biol 32, 1994, 523.
[23]. Mazumdar UK, Gupta M, Suryyendubikas M and Dilip M "Antitumor activity of *Hygrophila spinosa on* Ehrlich ascites carcinoma and sarcoma-180 induced mice". Ind J Exp Biol 35, 1997, 473-77.
[24]. Naigonkar AV, Burande MD, A manual of medical laboratory technology. Nirali Prakashan 2, 1996.
[25]. Suto T, Fukuda S, Moriya N, Watanabe Y, et al. Clinical study of biological reponse modifiers as maintenance therapy for hepatocellular carcinoma. Cancer Chemoth Pharm 33, 1994, 145-8.
[26]. Yoo YC, Saiki I, Sato K, Azuma I. MDP-Lys (L18), a lipophilic derivative of muramyl dipepetide, inhibits the metastasis of hematogenous and non hematogenous tumours in mice. Vaccine 12, 1994, 160-75.
[27]. Patel NP. Evaluation of antiarthritic potential of *Aristolochia bracteata* in rats (dissertation). Bangalore:Rajiv Gandhi University of Health Sciences 2006.
[28]. Sidram SA. Pharmacological evaluation of antiallergic activity of *Cynodon dactylon* (dissertation). Bangalore:Rajiv Gandhi University of Health Sciences 2007.
[29]. Kang NS, Moon EY, Cho CG, Pyo S. Immunomodulating effect of garlic component, allicin, on murine peritoneal macrophages. Nutr Res 21, 2001, 617-26.
[30]. Undem BJ. Pharmacotherapy of asthma. In: Laurence L, Lazo BJS, Parker KL, editors. Goodman and Gilman's: The pharmacological basis of therapeutics. The McGraw Hill Companies, Inc: New York; 11, 2005, 751-31.