Sethuramani A. *et al*. / *International Journal of Biological & Pharmaceutical Research.* 2014; 5(2): 150-155.

e- ISSN 0976 - 3651
Print ISSN 2229 - 7480

International Journal of Biological & Pharmaceutical Research
Journal homepage: www.ijbpr.com

IJBPR

ANTITUMOR ACTIVITY OF ETHANOLIC AND AQUEOUS EXTRACT OF *OLDENLANDIA UMBELLATA AND OLDENLANDIA CORYMBOSA* AGAINST DALTON'S ASCITIC LYMPHOMA IN MICE

Sethuramani A*, Jegadeesan M[1], Kavimani S[2]

*Lecturer, Department of Pharmacognosy, College of Pharmacy,
Madurai Medical College, Madurai-625020, Tamilnadu, India.
[1]Professor & Head, Dept. Environmental & Herbal Sci., Tamil University, Thanjavur-613010, Tamilnadu, India.
[2]Professor & Head, Dept. Pharmacology,
Mother Theresa Postgraduate and Research Institute of Health Sciences, Puducherry-605006, India.

ABSTRACT

Aims of the present study to evaluate the antitumor activity of ethanolic and aqueous extract of whole plant of *Oldenlandia umbellata*, & *Oldenlandia Corymbosa* on DAL model in Swiss albino mice. Evaluation of anti tumour activity of both *Oldenlandia umbellata*, & Olden Corymbosa on tumour growth and hosts survival time was made by the study of the following parameters : tumour volume, viable & non-viable cell count and life span of host. The results showed decreased in tumour volume and cell viability. Haematological studies revealed that the Hb count decreased in DAL treated mice, where as it was induced by the drug treated animals and showed an increase in Hb near to normal levels. The results suggested that, the extracts of whole plant of *Oldenlandia umbellata* & *Oldenlandia corymbosa* were exhibited significant antitumor activity of DAL bearing mice. It may be concluded that the whole plant extract of EEOU, EEOC, AEOU & AEOC significantly decreasing the tumor growth, this could be the reason for the increased percentage of life span of DAL bearing mice. These could be a source of natural antitumor that could have greater importance as therapeutic agent in preventing the tumor growth.

Key Words: *Oldenlandia umbellata*, & *Oldenlandia Corymbosa*, DAL model, Antitumor activity.

INTRODUCTION

Tumor is a mass of tissues which proliferate rapidly, spread throughout the body and may eventually cause death of the host (Mohan, 2006). By 2050 over 20 million new cancer cases and over 17 million cancer deaths are probable to occur in the world (American cancer society, 2006). Chemotherapy is an effective treatment against various types of cancer either singly or in combination with surgery and/or radiotherapy. However, chemotherapeutic effects of most of the drugs showed limited efficacy due to the development of various side effects. This fostered our attempts to evaluate some plant products against cancer as they are less likely to cause serious side effects. Many Indian spices are quoted to be useful in different types of cancer (Unnikrishnan MC & Kuttan R, 1990; Babu *et al*., 1995).

The traditional systems of Siddha and Ayurvedic medicine use this plant alone or in combination with other medicinal plants for the treatment of various diseases. A vast literature collection fails to produce a scientific evidence to prove the anti tumor activity of Oldenlandia umbellata and *Oldenlandia corymbosa*. Hence this study was planned to evaluate the effect of Ethanolic and Aqueous Extract of *Oldenlandia umbellata* and

Corresponding Author

A. Sethuramani
Email: mpharm76@gmail.com

Oldenlandia corymbosa against Dalton's Ascitic Lymphoma (DAL).

MATERIALS AND METHODS

Selection Grouping and Acclimatization of Laboratory Animal

Male Swiss albino mice (20-25gm) were procured from central animal house, and used throughout the study. They were housed in micro nylon boxes in a control environment (temp 25±2°C) and 12 h dark /light cycle with standard laboratory diet and water *ad libitum*. The study was conducted after obtaining institutional animal ethical committee clearance A.S/1573/2009/Ph.D/TU/26/PT. As per the standard practice, the mice were segregated based on their gender and quarantined for 15 days before the commencement of the experiment. They were fed on healthy diet and maintained in hygienic environment in our animal house (Unnikrishnan MC, Kuttan R, 1990).

Induction of Tumor

Various technique for induction of cancer in animals, viz. chemically induced (using DMBA/croton oil. etc) (Agarwal *et al*., 2009) virus induced, cell line induced (sarcoma – 180, ULCA fibro sarcoma and Jensen sarcoma, mouse lung fibroblast cells L-929, Dalton's Lymphoma Ascites (DLA), Ehrlich Ascites Carcinoma (EAC) (Becerra *et al*., 2006; David Apple Man *et al*., 1950; Chitra *et al*., 2009) methods have been used in experimental studies of anticancer activity.

Induction of cancer using DLA cells

Dalton's Lymphoma ascites (DLA) cells were supplied by Amala cancer research center, Trissur, Kerala, India. The cells maintained *invivo* in Swiss albino mice by intraperitoneal transplantation. While transforming the tumor cells to the grouped animal the DLA cells were aspirated from peritoneal cavity of the mice using saline. The cell counts were done and further dilution were made so that total cell should be 1×10^6, this dilution was given intraperitonealy. Allowed the tumor to grow in the mice for a minimum of seven days before starting the treatments.

Treatment Protocol

Swiss Albino mice were divided into seven groups of six animals each. All the animals in Six groups were injected with Dalton's Lymphoma Ascites (DLA) cells (1×10^6 cells per/ml/ mouse) (Nagineni Sujana *et al*., 2012) intraperitoneally, and the remaining one group is normal control.

Group 1 The normal control.

Group 2 The tumor control

Group 3 The positive control, was treated with injection fluorouracil at 20mg/kg body weight, i.p (Sathiyanarayanan *et al*., 2006).

Group 4 Served as the treatment control, which was treated with Aqueous Extract of *Oldenlandia umbellata* (AEOU) at 200 mg/kg body weight, in oral route.

Group 5 Served as the treatment control, which was treated with Ethanolic Extract of *Oldenlandia umbellata* (EEOU) at 200 mg/kg body weight, in oral route.

Group 6 Served as the treatment control, which was treated with Aqueous Extract of *Oldenlandia corymbosa* (AEOC) at 200 mg/kg body weight, in oral route.

Group 7 Served as the treatment control, which was treated with Ethanolic Extract of *Oldenlandia corymbosa* (EEOC) at 200 mg/kg body weight, in oral route.

In this study, drug treatment was given after 24 h of inoculation, once daily for 14 days. On day 14^{th} day after the last dose, all mice from each group were sacrificed by euthanasia. Blood was withdrawn from each mouse by retro orbital puncture method and the following Hematological parameters Serum enzyme and lipid profile & Derived parameters were checked (Jackson G & Jones G; Senthil *et al*., 2007; Gupta *et al*., 2004; Spiridon KE, 2006).

Hematological parameters

WBC count

The WBC count was found to be increased in cancer control, when compared with normal and treated tumor-bearing mice. The WBC count were found to decrease significantly in animals treated with plant extract when compared with cancer control, indicating that the antitumor nature of the extract (Santhosh Kumar *et al*., 2007).

RBC and Hb

RBC and Hb content decreases with tumor bearing mice when compared with Normal control mice.

Platelets

In Hodgkin lymphoma, increase in platelet count often reported in laboratory finding. Hence, this parameter in the study is investigated (Jacqueline *et al*., 1998).

Packed cell volume

In the case of anemia the packed cell volume decreases.

Serum Enzyme and Lipid Profile

Total Cholesterol and Triglycerides

Abnormal blood lipid profile has been associated with cancer. In Hodgkin lymphoma, high cholesterol level and low triglyceride level has been reported and investigated in this parameter study (Ronald AS, 1995).

Liver Enzymes (AST, ALT, ALP)

Abnormal liver function seen in patient with Hodgkin lymphoma (Viroj wiwanikit, 2001) that these liver enzyme levels markedly increase in tumor bearing mice. AST, ALT, ALP is an enzyme mainly derived from the liver, bones and in lesser amount from intestines,

placenta, kidneys and leukocytes. An increase in AST, ALT and ALP levels in the serum are frequently associated with the variety of disease (Intyre MC & Rosalki S, 1991). AST, ALT and ALP comprise a group of enzyme that catalyzes the phosphate esters in an alkaline environment, generating an organic radical and inorganic phosphate.

Markedly elevated serum AST, ALT and ALP, hyperalkaline-phosphatasemia, is seen predominantly with more specific disorders; including malignant biliary cirrhosis, hepatic lymphoma and sarcoidosis (Jahan *et al*., 2008).

Derived Parameters

Body weight

All the mice were weighed, from the beginning to 15th day of the study. Average increase in body weight on the 15th day was determined.

Percentage increase in life span (ILS)

% ILS was calculated by the following formulae

$$\frac{\text{Life span of treated group}}{\text{Life span of control group}} - 1 \times 100$$

All biochemical investigations were done by using OBAS MIRA PLUS-S Auto analyzer from Roche Switzerland.

Hematological test are carried out in COBAS MICROS OT 18 from Roche.

Newly added Hi-Tech instruments MAX MAT used for an auto analyzer for all biochemical investigations in blood sample.

Evaluation of Clinical Parameters

Cancer cell count

The fluid (0.1ml) from the peritoneal cavity of each mouse was withdrawn by sterile syringe and diluted with 0.8 ml of ice cold Normal saline or sterile Phosphate Buffer Solution and 0.1 ml of tryphan blue (0.1 mg/ml) and total numbers of the living cells were counted using heamocytometer (Mary *et al*., 1994).

$$\text{Cell count} = \frac{\text{No of cells Dilution}}{\text{Area} \times \text{Thickness of liquid film}}$$

Table 1. Effect of AEOU, EEOU, AEOC and EEOC on Hematological Parameters

Treatment	Total WBC Cells /mlx10^3	RBC Count Millon/cumm	Hb gm/dl	PCV in %	Platelets Lakhs/cumm
G1	9.88 ±1.64	4.88±0.96	12.05 ±1.68	15.25±2.35	3.56±0.95
G2	14.62 ±3.12a**	2.32±0.35a**	7.15 ±0.90a**	30.62±3.30a**	1.42±0.78a**
G3	11.28 ±1.23b**	4.25±0.81b**	11.5 ±1.35b**	17.32±1.72b**	2.55±0.86b**
G4	12.76±2.18b**	2.90±0.48b**	9.38±0.98b**	23.12±2.23b**	1.96±0.40b**
G5	12.98 ±2.64b**	3.02±0.52b**	9.69±1.08b**	22.95±2.40b**	2.30 ±0.72b**
G6	13.15 ±3.05b**	3.16±0.66b**	10.60±1.26b**	23.05±2.52b**	2.12 ±0.61b**
G7	12.26 ±3.55b**	3.22±0.72b**	10.98±1.45b**	22.78±2.24b**	2.05 ±0.56b**

G1 – Normal Control, G2 – Cancer Control, G3 – Positive control, G4 – Treatment control (AEOU-200mg/kg), G5 – Treatment control (EEOU-200mg/kg), G6 – Treatment control (AEOC-200mg/kg), G7 – Treatment control (EEOC-200mg/kg).

All values are expressed as mean ± SEM (n=6).

a** – Values are significantly different from control (G1) $P < 0.001$

b** – Values are significantly different from cancer control (G2) $P < 0.001$

Table 2. Effect of AEOU, EEOU, AEOC and EEOC on Serum Enzymes and Lipid Proteins

Treatment	Cholesterol (mg/dl)	TGL (mg /dl)	AST (U/L)	ALT (U/L)	ALP (U/L)
G1	100.12±5.68	126.25±2.58	38.56 ±1.26	34.36 ±1.45	128.48 ±3.25
G2	152.32±7.22a**	215.08±5.68a**	92.52±2.78a**	68.45±3.22a**	245.65±7.37a**
G3	116.15±4.45b**	158.46±3.58b**	52.34 ±1.88b**	42.32±2.08b**	162.55±4.30b**
G4	122.48±4.95b**	168.15±3.39b**	71.25 ±2.20b**	50.05±2.78b**	180.45±4.84b**
G5	124.88±4.98b**	162.68±2.98b**	68.22±1.95b**	48.50 ±2.40b**	182.40±4.22b**
G6	120.12±4.23b**	170.15±3.05b**	64.58±1.20b**	46.34 ±2.09b**	178.30±4.08b**
G7	121.30±4.08b**	164.75±3.12b**	65.18±1.32b**	49.15 ±2.46b**	176.24±4.15b**

G1 – Normal Control, G2 – Cancer Control, G3 – Positive control, G4 – Treatment control (AEOU-200mg/kg), G5 – Treatment control (EEOU-200mg/kg), G6 – Treatment control (AEOC-200mg/kg), G7 – Treatment control (EEOC-200mg/kg).

All values are expressed as mean ± SEM (n=6).

a **– Values are significantly different from control (G1) $P < 0.001$

b** – Values are significantly different from cancer control (G2) $P < 0.001$

Table 3. Effect of AEOU, EEOU, AEOC and EEOC on the Life Span, Body Weight and Cancer Cell Count of Tumor Induced Mice

Treatment	Number of animals	% ILS Life span	Body weight in grams	Cancer cell count ml X 106
G1	6	>>30 days	2.26±0.52	-
G2	6	50%	7.88±0.91a**	2.68±0.42a**
G3	6	90%	3.82±0.23b**	1.30±0.34b**
G4	6	70%	4.15±0.65b**	1.98±0.56b**
G5	6	72%	4.50±0.74b**	1.60±0.35b**
G6	6	76%	4.12±0.53b**	1.66±0.30b**
G7	6	74%	4.32±0.66b**	1.75±0.38b**

G1 – Normal Control, G2 – Cancer Control, G3 – Positive control, G4 – Treatment control (AEOU-200mg/kg), G5 – Treatment control (EEOU-200mg/kg), G6 – Treatment control (AEOC-200mg/kg), G7 – Treatment control (EEOC-200mg/kg)
All values are expressed as mean ± SEM (n=6).
a** – Values are significantly different from control (G1) at $P < 0.001$
b** – Values are significantly different from cancer control (G2) at $P < 0.001$

RESULTS

Effect on Tumor Growth

In the DLA tumor control group, the average life span of animal was found to be 50% whereas AEOU, EEOU, AEOC and EEOC at a dose of 200 mg/kg body weight increase the life span to 70%, 72%, 76% and 74% respectively. These values were significant. However the average life span of 5- FU treatment was found to be 90%, indicating its potent antitumor nature. The antitumor nature of AEOU, EEOU, AEOC and EEOC was evidenced by the significant reduction in percent increase in body weight of animal treated with AEOU, EEOU, AEOC and EEOC at the dose of 200 mg/kg body weight when compared to DLA tumor bearing mice.

It was also supported by the significant reduction in packed cell volume and viable Tumor cell count in both the extracts of treatment when compared to the DLA tumor control (Table: 3)

Effect on Hematological Parameters

As shown in (Table: 1) RBC, HB Platelets were decreased and WBC count was significantly increased in the DLA control group compared to the normal control group. Treatment with AEOU, EEOU, AEOC and EEOC at the dose of 200 mg/kg significantly increases the Hb content, RBC, Platelets and significantly decreased the WBC count to about normal level. All these results suggest the anticancer nature of the extract. However, the standard 5-FU at the dose of 20 mg/kg body weight produced better result in all these parameters.

Effect on Biochemical Parameters

The inoculation of DLA cells caused significantly increase in the level of Total Cholesterol, Aspartate amino Transferase, Alanine, amino Transferase, Alkaline Phosphatase in the tumor control animals(G2), when compared to the normal group. The treatment with AEOU, EEOU, AEOC and EEOC at the dose of 200 mg/kg body weight reversed these changes towards the normal level (Table-2) All the values were found to be significant. The treatment with standard 5- FU also gave similar results.

DISCUSSION AND CONCLUSION

The alternative system of medicines like Ayurvedic, Siddha, Unani and other tribal folklore medicines have significantly contributed to the health care of the population of India. Today these systems are not only complementary but also competitive in the treatment of various diseases. Plants have served as a good source of antitumor agents. Several studies have been conducted on herbs under a multitude of Ethanobotanical grounds. A large number of plants possessing anticancer properties have been documented (Jasmine *et al*., 2008; Abeeu LA & Abeeu RR, 1979; Ramalingam Radha *et al*., 2008; Dauod *et al*., 2004; Rajkapoor *et al*., 2004; Ashok Kumar Durairaj *et al*., 2009).

Plants of *Oldenlandia umbellata* and *Oldenlandia corymbosa* were traditionally used in the treatment of tumors. The present investigation was carried out to evaluate the antitumor activity of Ethanolic and Aqueous extracts of *Oldenlandia umbellata* and *Oldenlandia corymbosa* in DLA tumor bearing mice. The AEOU, EEOU, AEOC and EEOC treated animals at the doses of 200 mg/kg significantly inhibited the tumor volume, packed cell volume, tumor (viable) cell count and brought back the hematological parameters to more or less normal levels.

In DLA tumor bearing animals a regular rapid increase in ascitic tumor volume was observed. Ascitic fluid is the direct nutritional source for tumor cells and a rapid increase in ascitic fluid with tumor growth would be a means to meet the nutritional requirement of tumor cells (Prasad SB & Giri A, 1994). Treatment with AEOU, EEOU, AEOC and EEOC inhibited the tumor volume, viable tumor cell count and increased the life span of the tumor bearing mice. The reliable criteria for judging the value of any anticancer drug are the prolongation of the lifespan of animals (Clarkson BD & Burchenal JH, 1965).

It may be concluded that AEOU, EEOU, AEOC and EEOC by decreasing the nutritional fluid volume and arresting the tumor growth increases the life span of DLA bearing mice. Thus AEOU, EEOU, AEOC and EEOC have antitumor activity against DLA bearing mice.

Usually, in cancer chemotherapy the major problems that are being encountered are myelo suppression and anemia (Price VE, Greenfield RE, 1958; Hogland HC, 1982). The anemia encountered in tumor bearing mice is mainly due to reduction in RBC or Hb and this may occur either due to iron deficiency or due to hemolytic or myelopathic conditions (Fenninger LD & Mider GB, 2004). Treatment with AEOU, EEOU, AEOC and EEOC brought back the (Hb) content; RBC and WBC count more or less to normal levels significantly. This clearly indicates that AEOU, EEOU, AEOC and EEOC possess protective action on the haemopoietic system.

It was reported that the presence of tumor in the human body or in the experimental animals is known to affect many functions of the liver. The significantly elevated levels of total cholesterol, TGL, AST, ALT, ALP in serum of tumor inoculated animal indicated liver damage and loss of functional integrity of cell membrane. The significant reversal of these changes towards the normal by AEOU, EEOU, AEOC and EEOC treatments.

In the present study, the biochemical examination of DLA inoculated animals showed marked changes indicating the toxic effect of the tumor. The normalization of these effects observed in the serum treated with AEOU, EEOU, AEOC and EEOC possesses significant antitumor and hepatoprotective effect of the extracts.

REFERENCES

Abeeu LA, Abeeu RR. Changes induced by Ehrlich Ascites carcinoma in hepatic Fumarase and Aconitase activities. RJ (Brazil). 1979. p. 1536.

Agarwal RC, Rachana Jain, Wasim Raju, Ovais M. Anti-Carcinogenic effects of Solanum lycopersicum fruit extract on Swiss albino and C57B1 Mice. *Asian. Pacific. J. Cancer Prev*. 2009; 10:379-382.

American cancer society (2006). A biotechnological company dedicated to cancer treatment.

Ashok Kumar Durairaj, TamilSelvan Vaiyapuri, Upal Kanti Mazumder, Malaya Gupta. Antineoplastic and antioxidant activities of Oxystelma esculentum on Swiss albino mice bearing Ehrlich's ascites carcinoma. *Pharmaceutical Biology*. 2009; 47(3):195-202.

Babu TD, Kuttan G, Padikkala J. Cytotoxic and Antitumour activity of certain taxa of Umbelliferae with special reference to Centella asiatica (L.) *Urban. Journal of Ethnopharmacology*. 1995; 48: 53-57.

Becerra DP, Castro FO, Alves APNN, Dessoa C, Moraes MO, Silveria ER, Lima MAS, Elmiro FJM, Costa-Lotufo LV. In vivo growth – inhibition of sarcoma 180 by piplartine and piperine two alkaloid amides from piper. *Brazilian Journal of Medical and Biological Research*. 2006; 39(6):801-807.

Chitra V, Shrinivas Sharma,Nandu Kayande. Evaluation of Anticancer activity of Vitex negundo study. *International Journal of Pharm Tech Research*. 2009; 1(4):1485-1489.

Clarkson BD, Burchenal, JH. Preliminary screening of antineoplastic drugs. *Prog. Clin. Cancer*. 1965; 1:625-9.

Dauod M, Nihat D, Hatice G, Muharren B. Antitumor activity of Ethanol Extract of Nigella sativa seeds. *Bilogia Bratisiava*. 2004; 59:735-740.

David Apple Man, Edwin R, Skavinski Abraham M, Stein. Catalase Studies on Normal and Cancerous rats. *Cancer Research*. 1950; 10:498-505.

Fenninger LD, Mider GB. Energy and nitrogen metabolism in cancer. *Adv. cancer Res*. 1954; 2:229-253.

Gupta M, Mazumder UK, Sambath KR, Siva Kumar T, Vamsi MLM. Antitumor activity and antioxidant status of Caesalpinia bonducella against Ehrlich ascites carcinoma in Swiss albino mice. *J Pharmacol Sci*. 2004; 94:177–184.

Hogland HC. Hematological complication of cancer chemotherapy. *Semin.Oncol*. 1982; 9:95-102.

Intyre MC, Rosalki S. Biochemical investigations in the management of Liver Disease. In: Oxford Text book of Clinical Hepatology, Oxford University Press, Chennai, 1991.p. 293-309.

Jackson G, Jones G. Leukemia. In: clinical pharmacy and therapeutics.4th ed. (Roger, W and Clive, eds,) Churchill Livingstone Elsevier; New York.p.715–730.

Jacqueline MH, Darius JN, Mathew JM, Ronald DB, Blood Lipid Profile in Children's with Acute Lymphoblastic Leukemia. *Cancer.* 1998; 83:379-384.

Jahan AK, Sulthan S, Rowshanul H, Kumar S, Mosin A. Antineoplastic activity of Bis – Tyrosinediaqu Ni (II) against Ehrlich Ascites Carcinoma. *Dhaka Univ. J. Pharm. Sci*. 2008; 7:33–37.

Jasmine M, Ali MM, Biswas AK, Habib MR, Khanam JA. Antineoplastic activity of Nickel (II) – Cystine complex against Ehrlich Ascites Carcinoma in Swiss Albino mice. *Medical Journal of Islamic World Academy of Science*. 2008; 16:135–142.

Mary KT, Kuttan G, Kuttan K. Partial purification of Tumor reducing principle from Helicanthis elasticus. *Cancer Letter*. 1994; 81:53-57.

Mohan H. Textbook of Pathology. Jaypee Brothers Medical Publishers (P) Ltd; New Delhi: 2006: 445.

Nagineni Sujana, Santhanalakshmi R, Venketela V, Meenakshi sundaram M, Brindha P.Antitumour Potential of Passiflora Incarnatall against ehrlich ascites carcinoma. *International Journal of Pharmacy and Pharmaceutical sciences.* 2012; 4:17-20.

Prasad SB, Giri A. Anti tumor effect of cisplatin against Murine Ascites Dalton's lymphoma. *Indian J. Exp. Biol*. 1994; 32:155-62.

Price VE, Greenfield RE. Anemia in cancer. *Adv. Cancer Res*. 1958; 5:199-200.

Rajkapoor B, Jayakar B, Murugesh N. Antitumor activity of Indigofera asphalathoides on Ehrlich Ascites Carcinoma in mice. *Indian Journal of Pharmacology*. 2004; 36: 38-40.

Ramalingam Radha, Subramaniyam, Kavimani, Velayudham, Ravichandran. Antitumor activity of Methanolic extract of Plumeria Alba Linn leaves against Dalton's Lymphoma ascites in mice. *International Journal of Health Research*. 2008; 1(2):79–85.

Ronald AS. Disease of White blood cells. In, Wildman's Clinical Interpretation of Laboratory Tests, 10th ed, Jaypee Press, New Delhi, 1995. p.164.

Santhosh Kumar H, SenthilKumar N, Reghu CH. Anti tumor activity of Methanolic extract of Hypericum hookerianum on EAC Cell line in Swiss albino mice. *J. Pharmacological Sci*. 2007; 103:354-359.

Sathiyanarayanan L, Shinnathambi Arulmozi, Chidhambaranathan,N. Anti Carcinogenic activity of Leptadenia reticulate against Dalton's ascitic lymphoma. *Iranian Journal of Pharmacology and Toxicology*. 2006; 6: 133–136.

Senthil KN, Shrishailappa B, Santosh Kumar H, Ashok G. Antitumor activity and antioxidant activity of the Methanolic Extract of bark against Daltons Lymphoma ascites induced Ascitic and solid tumor in mice. *Indian journal of Pharmaceutical Sciences*. 2007; 3:12–23.

Spiridon KE. Terrestrial Plant –Derived Anticancer Agents and Plants used in Anticancer Research. *Crit. Rev. Plant Sci*. 2006; 25: 79-113.

Unnikrishnan MC, Kuttan R. Tumor reducing and Anti-Carcinogenic activity of selected species. *Cancer Letter*. 1990; 51: 85-89.

Viroj wiwanikit. High Serum alkaline Phosphatase Level in Hospitalized Patient. *BMC Family Practice*. 2001; 10:1861, 1471-2296.