

ANTIOXIDANT AND ANTITUMOR ACTIVITIES OF *POLYALTHIA SIMIARUM* (BUCH.-HAM. EX HOOK. F. & THOMSON) HOOK. F. & THOMSON

Selina KABIR¹, Ronok ZAHAN², Ashik MOSADDIK², Abdullah Mohammad Sarwaruddin CHOWDHURY¹, Choudhury Mahmood HASAN³, Mohammad Abdur RASHID^{3*}

¹University of Dhaka, Faculty of Engineering and Technology, Department of Applied Chemistry & Chemical Engineering, Dhaka-1000, BANGLADESH

²BRAC University, Department of Pharmacy, Dhaka, BANGLADESH

³University of Dhaka, Faculty of Pharmacy, Department of Pharmaceutical Chemistry, Dhaka-1000, BANGLADESH

Abstract

The present study has been designed to investigate the antioxidant and antitumor activities of ethyl acetate (EA) extract of stem bark of *Polyalthia simiarum* (Annonaceae). Antioxidant potential of the EA extract was evaluated in vitro by DPPH (1,1-diphenyl-2-picrylhydrazyl) scavenging assay method. The EA extract showed free radical scavenging activity with IC₅₀ value of 24.08 ± 0.30 µg/mL. The antitumor activity of the extract was determined against Ehrlich ascites carcinoma (EAC) in mice at 25 mg/kg and 50 mg/kg body weight intraperitoneally. Significant (p<0.001) increase of survival time by 25 ± 0.57 and 27 ± 0.40 days by the EA extract treated tumor bearing mice was confirmed with respect to the control group (22 ± 0.12 days). Hematological studies revealed that the hemoglobin (Hb) content was decreased in EAC treated mice whereas restoration to close to normal levels was observed in extract treated animals. There was a significant (p<0.001) decrease in RBC and increase in WBC counts in extract treated animals when compared to EAC affected animals. Therefore, the EA extract of *P. simiarum* was capable to exhibit moderate antioxidant and antitumor activities. This is the first report of antioxidant and antitumor potential of *P. simiarum*.

Key words: Antitumor, Ehrlich ascites carcinoma, Free radical, *Polyalthia simiarum*

Polyalthia simiarum (Buch.-Ham. ex Hook. F. & Thomson) Hook. F. & Thomson'un Antioksidan ve Antitümör Aktivitesi

Bu çalışmada *Polyalthia simiarum* (Annonaceae)'un gövde kabuğunun etil asetatlı ekstresi (EA) antioksidan ve antitümör aktiviteleri bakımından incelenmiştir. EA ekstresinin antioksidan potansiyali in vitro olarak DPPH (1,1-difenil-2-pikrilhidrazil) radikali süpürücü etki ile ölçülmüştür. EA ekstresi 24.08 ± 0.30 µg/mL IC₅₀ değeri ile serbest radikal süpürücü aktivite göstermiştir. Ekstrenin antitümör aktivitesi intraperitoneal olarak 25 mg/kg ve 50 mg/kg vücut ağırlığında farede Ehrlich ascites karsinoma (EAC)'ya karşı belirlenmiştir. EA ekstresi uygulanan tümör taşıyan farelerde hayatta kalma süresi kontrol grubuna göre (22 ± 0.12 gün) 25 ± 0.57 ve 27 ± 0.40 gün ile belirgin (p<0.001) bir artış göstermiştir. Hematolojik çalışmalar hemoglobin (Hb) içeriğinin EAC farelerde azaldığını, bununla birlikte ekstre verilen hayvanlarda normal düzeylere yakın hale geldiğini göstermiştir. EAC taşıyan hayvanlarla karşılaştırıldığında, ekstre uygulanan hayvanlarda WBC değerinde artma ve RBC değerinde belirgin (p<0.001) bir azalma vardır. Bu sonuçlara bağlı olarak, *P. simiarum*'un EA ekstresi orta düzeyde antioksidan ve antitümör aktiviteye sahiptir. Bu çalışma *P. simiarum*'un antioksidan ve antitümör etkilerinin incelendiği ilk çalışmadır.

Anahtar kelimeler: Antitümör, Ehrlich ascites karsinoma, Serbest radikal, *Polyalthia simiarum*

*Correspondence: E-mail: rashidma@univdhaka.edu; rashidma@du.ac.bd; Tel:+880-2-8653887

INTRODUCTION

The morbidity and mortality of cancer, the second leading cause of death worldwide next to cardiovascular diseases, is characterized by uncontrolled cellular growth, local tissue invasion, and distant metastases (1). Free radicals, one of the major causes for the conversion of normal cell to cancerous cells, are generated as a consequence of a number of endogenous metabolic processes involving redox enzymes and bioenergetic electron transfer and exposure to a plethora of exogenous chemicals (2). In normal metabolic condition, oxidant and antioxidant levels are maintained in balance within humans for sustaining optimal physiological conditions (3). However, overproduction of free radical and reactive oxygen species (ROS) would assault on important biological molecules such as DNA, protein or lipid leading to many degenerative processes, such as cancer, Alzheimer's disease, arthritis and ischemic reperfusion (4). More and more evidences suggest that this potentially cancer-inducing oxidative damage might be prevented or limited by antioxidants which may mediate their effect by directly reacting with ROS, quenching them or chelating the catalytic metal ions (5). It has been shown that antioxidant rich diet can reduce oxidative damage to DNA, thus preventing a critical step in the onset of carcinogenesis and the impact of antioxidant on mutagenesis and carcinogenesis has been well established (6,7). Moreover, the increase of cancer incidence and lack of appropriate anticancer drugs have forced scientists to pharmacological and chemical investigation of anticancer agents from medicinal plant (8). The worldwide upsurge in the use of herbal preparation and medicinal plant along with their isolated active compounds has provided one of the most important sources for pharmaceutical industry for lead compounds. Furthermore, over a 100 new products are in clinical development, particularly as anti-cancer and anti-infective agents (9). Although, the mechanism of interaction between phytochemicals and cancer cells has been studied extensively and augmented the interest of pharmacological evaluation of various plants used in Bangladeshi traditional systems of medicine (10).

The genus *Polyalthia* (Annonaceae) comprises about 70 species, out of which only eight are indigenous to the Indian subcontinent (11). *P. simiarum* (Buch.-Ham. ex Hook. f. & Thomson) Hook. f. & Thomson, locally known as Arjan, is a very tall tree. In the traditional system of medicine, the plants of this genus are used as a bitter tonic, abortifacient, febrifuge, cure for scorpion stings, hypertension and as respiratory stimulant (12). Biological evaluations of *Polyalthia* species have shown them to exhibit cytotoxic, antimicrobial (13), anticancer (14), antimalarial (15) and HIV-inhibitory (16) activities. The plant *P. simiarum* is known to exhibit antimicrobial and cytotoxic (17) activities. Previous phytochemical investigations with *Polyalthia* species revealed to contain alkaloids (azafluorene, polylongine and aporphine), steroid, diterpenoids and pentacyclic triterpenes (18). Very recently the petroleum ether extract of stem bark of *P. simiarum* led to the isolation of a bisnor-type clerodane diterpenoid, 2-oxo-14,15-bisnor-3,11*E*-kolavadien-13-one and three clerodane derivatives, kolavenic acid, 16 β -hydroxycleroda-3,13(14)*Z*-dien-15,16-olide and 16-oxocleroda-3,13(14)*E*-dien-15-oic acid (19). As not enough phytochemical and biological studies have been carried out with *P. simiarum*, the present study was conducted to evaluate the antioxidant potential by DPPH and antitumor activity of ethyl acetate extract of the stem bark of *P. simiarum* against Ehrlich ascites carcinoma (EAC) in mice.

EXPERIMENTAL

Plant material

The stem bark of *Polyalthia simiarum* (Buch.-Ham. ex Hook. f. & Thomson) Hook. f. & Thomson was collected from Mirpur, Dhaka in the month of June 2008 and identified by Mr. Sarder Nasir Uddin, Scientific Officer, Bangladesh National Herbarium, Dhaka, where a voucher specimen (DACB-34201) representing this collection has been deposited.

Chemicals

Sodium chloride, propylene glycol, trypan blue, methylene blue, methyl violet and sodium sulfate, were purchased from Merck Limited, Mumbai, India. All other chemicals and reagents used were of highest analytical grade.

Preparation of extract

The air dried and powdered plant material (700 g) was extracted in a Soxhlet apparatus with ethyl acetate (60-80°C). The extract was filtered through a fresh cotton plug followed by Whatman number 1 filter paper. The filtrate was then concentrated with a Buchii rotavapor at low temperature and pressure to afford ethyl acetate extract (EA, 7.52 g approx.).

Preliminary phytochemical investigation

The extract was subjected to qualitative chemical investigation for the identification of different phytoconstituents like sterols, glycosides, saponins, carbohydrates, alkaloids, flavonoids, tannins, proteins and terpenoids (20).

Animals

Swiss albino mice (25-30 g) of both sexes were used for assessing the biological activity. The animals were maintained under standard laboratory conditions and had free access to food and water *ad libitum*. The animals were allowed to acclimatize to the environment for 7 days prior to experimental session. The animals were divided into different groups, each consisting of five animals which were fasted overnight prior to the experiments. The experiments with the animals were performed in accordance with guidelines of the Institutional Animal Ethics Committee, Department of Applied Chemistry & Chemical Engineering, University of Rajshahi, Rajshahi, Bangladesh.

Acute toxicity

The acute oral toxicity of the plant extract in Swiss albino mice was studied as per established protocol (21).

In vitro Antioxidant Activity

Free radical scavenging activity by 1,1-diphenyl-2-picryl-hydrazyl (DPPH) assay

The free radical scavenging activity of EA extract, based on the scavenging of the stable 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical, was determined by the method described by Braca *et al.* (22). The percentage inhibition was calculated from $[(A_0 - A_1)/A_0] \times 100$, where A_0 is the absorbance of the control, and A_1 is the absorbance of the extract/ standard. IC_{50} value was calculated by plotting a graph of concentration ($\mu\text{g/mL}$) versus % inhibition.

In vivo Antitumor Activity

Transplantation of tumor

Ehrlich ascites carcinoma (EAC) cells were obtained from Indian Institute of Chemical Biology (IICB), Kolkata, India. The EAC cells were maintained *in vivo* in Swiss albino mice by intraperitoneal transplantation of 2×10^6 cells per mouse after every 10 days. Ascitic fluid was drawn out from EAC tumor bearing mouse at the log phase (days 7-8 of cell implantation) of the tumor cells. Each animal received 0.1 mL of tumor cell suspension containing 2×10^6 tumor cells intraperitoneally.

Treatment schedule

60 Swiss albino mice were divided into five groups (n =12) and given food and water *ad libitum*. All the animals in each group except Group-I received EAC cells (2×10^6 cells/mouse, i.p.). This was taken as day '0'. Group-I served as normal saline control (5 mL/kg b.w., i.p.) and

Group-II served as EAC-treated control. After 24 h of EAC transplantation, Group-III and Group-IV received EA extract of *P. simiarum* stem bark at 50- and 25 mg/kg b.w., i.p. for nine consecutive days, respectively. Group-V received reference drug Bleomycin (0.3 mg/kg b.w., i.p.) for nine consecutive days (23). After 24 hours of last dose and 18 h of fasting, 6 animals from each group were sacrificed by cervical dislocation to measure antitumor and hematological parameters and the rest were kept with food and water *ad libitum* to check percentage increase in life span of the tumor bearing host. The antitumor activity of the extract of *P. simiarum* was measured in EAC-treated animals with respect to the following parameters.

Determination of tumor volume and weight

The mice were dissected and the ascitic fluid was collected from the peritoneal cavity. The volume was measured by taking it in a graduated centrifuge tube and weighed immediately.

Tumor cell count

The ascitic fluid was taken in a WBC pipette and diluted 100 times with normal saline and then a drop of the diluted cell suspension was placed on the Neubauer's counting chamber and the number of cells in the 64 small squares was counted.

Viable/nonviable tumor cell count

The viability and non viability of the cells were checked by trypan blue assay with the help of microscope. The cells were stained with trypan blue (0.4% in normal saline) dye. The cells that did not take up the dye were viable and those that took the dye were nonviable. These viable and nonviable cells were counted using the following equation:

$$\text{Cell count} = (\text{Number of cells} \times \text{dilution factor}) / (\text{Area} \times \text{thickness of liquid film})$$

Determination of median survival time and percentage increase in life span

The mortality was monitored by recording percentage increase in life span (%ILS) and median survival time (MST) (24).

Body weight

Body weights of the experimental mice were recorded both in the treated and control groups at the beginning of the experiment (day 0) and sequentially on every 9th day during the treatment period.

Hematological parameters

Collected blood was used for the estimation of hemoglobin (Hb) content, red blood cell (RBC) and white blood cell count (25).

Statistical analysis

Antitumor data are expressed as mean \pm S.E.M. (n = 6 mice per groups). Statistical significance (p) calculated by Student's t test. $P < 0.001$ and < 0.05 were considered to be statistically significant.

RESULTS AND DISCUSSION

The phytoconstituents present in the EA extract of *P. simiarum* were identified by various chemical tests which showed the presence of alkaloids, terpenoids, phenolic and flavonoid compounds and steroids (Table 1).

The acute toxicity study was conducted to establish the therapeutic index, i.e., the ratio between the pharmacologically effective dose and the lethal dose on the same strain and species.

The extract of *P. simiarum* was safe up to a dose of 1000 mg/kg b.w. (p.o.) body weight. Behavior of the animals was closely observed for the first 3h then at an interval of every 4h during the next 48h. The extract did not cause mortality in mice during 48h of observation but little behavioral changes, locomotors ataxia, diarrhea and weight loss were observed. Food and water intake had no significant difference among the group studied.

Table 1. Result of chemical group tests of the EA extract of *Polyalthia simiarum*.

Extract	Triterpene	Diterpene	Flavonoid	Phenol	Sterol	Alkaloid
<i>Polyalthia simiarum</i> (EA)	+	+++	+++	++	+++	++

EA: Ethyl acetate extract; (+): Present; (-): Absent; (+++): Reaction intensity is high; (++): Reaction intensity is medium; (+): Reaction intensity is normal.

The percentage (%) scavenging of DPPH free radical was found to be concentration dependent, i.e. concentration of the extract between 15.62-500 µg/ml greatly increasing the inhibitory activity (Figure 1). The IC₅₀ value of the EA extract was 24.08± 0.30µg/mL, while ascorbic acid showed the value of 12.30 ± 0.11 µg/mL.

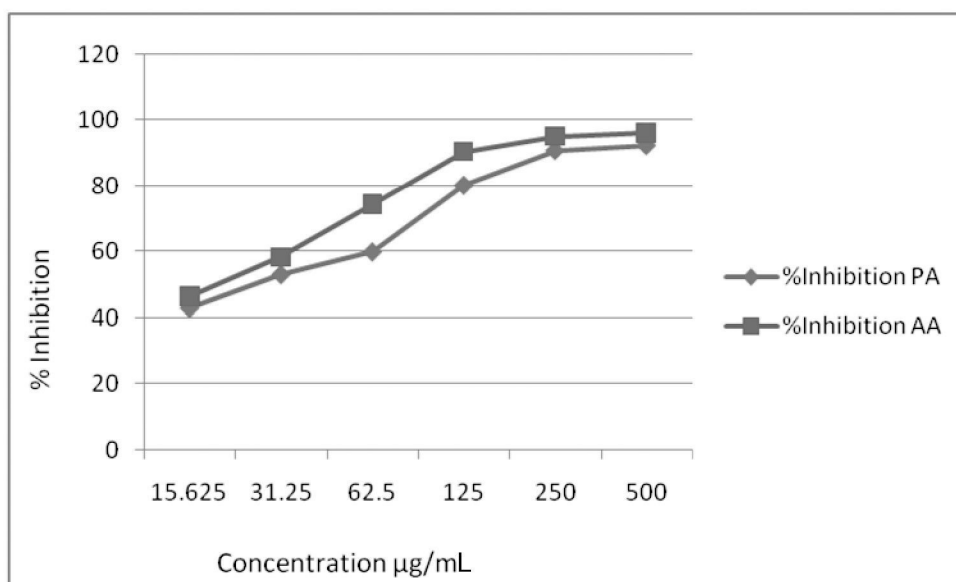


Figure 1. Free radical scavenging activity of different concentrations of EA extract of *P. simiarum* by DPPH radicals (PA: *Polyalthia*, AA: Ascorbic acid).

Antitumor activity of extract against EAC tumor bearing mice was assessed by the parameters such as tumor volume, tumor weight, cell count (viable and non viable), mean survival time and % increase of life span. The results are shown in Table 2. The tumor volume, tumor weight and viable cell count were found to be significantly ($p<0.001$) increased and non-viable cell count was significantly ($p<0.001$) low in EAC-treated control animals when compared with normal control animals. Administration of EA extract at dose of 50- and 25 mg/kg b.w. significantly ($p<0.05$) decreased the tumor volume, tumor weight and viable cell count. Furthermore, the median survival time was increased to 27 ± 0.40 and 25 ± 0.57 (% ILS = 22.12 and 13.63) on administration of crude extract at 50- and 25 mg/kg b.w., respectively. All these results clearly indicate that the EA extract had capacity to inhibit the growth of solid tumor induced by EAC cell line in experimental animals.

Hematological parameters (Table 3) of tumor bearing mice on 14 day were found to be significantly altered compared to the normal group. The total WBC count was found to be

increased with a reduction of Hb content of RBC. The total number of RBC showed a modest change. At the same time the EA extract at 50- and 25 mg/kg b.w. restored all the altered hematological parameters to almost close to normal.

Table 2. Effects of the EA extract of *Polyalthia simiarum* on tumor volume, tumor weight, mean survival time (MST), percentage increase life span (% ILS), viable and non-viable tumor cell count in EAC bearing mice.

Parameter	EAC control	EA extract (50mg/Kg b.w.)	E A extract (25mg/Kg b.w.)	Bleomycin
Tumor volume (mL)	3.2 ± 0.21	0.79 ± 0.34*	1.70 ± 0.028**	0.52 ± 0.21*
Tumor weight (g)	3.70 ± 0.24	1.22 ± 0.21*	2.31 ± 0.026**	0.51 ± 0.11*
MST (days)	22 ± 0.12	27 ± 0.40	25 ± 0.57	44.6 ± 0.12
% ILS	00.0	22.12	13.63	96.81
Viable cell (× 10 ⁷ cell/mL)	8.1 ± 0.22	0.23 ± 0.05*	0.56 ± 0.024**	0.5 ± 0.05*
Non-viable cell (×10 ⁷ cell/mL)	0.5 ± 0.24	0.76 ± 0.54*	1.13 ± 0.049**	3.3 ± 0.05*
Total cell (× 10 ⁷ cell/mL)	8.6 ± 0.15	0.99 ± 0.21*	1.69 ± 0.026**	3.8 ± 0.05*
Viable %	94.18	23.23	33.13	13.15
Non-viable %	5.82	76.76	66.86	86.85

Each point represent the mean ± SEM. (n=6 mice per group), **p*<0.05 statistically significant when compared with EAC control group.

To determine the efficacy of natural antioxidants either as pure compounds or as plant extract, a great number of *in vitro* methods have been developed in which antioxidant compounds act by several mechanisms. DPPH is a stable free radical that accepts an electron or hydrogen radical to become a stable diamagnetic molecule (26) and is usually used as a substrate to evaluate the antioxidant activity of a compound (27). Based on the data obtained from this study, DPPH radical scavenging activity of EA extract (IC₅₀ 24.08 ± 0.30 µg/mL) was lower than the standard (IC₅₀ 12.30 ± 0.11 µg/mL). It was revealed that the EA extract demonstrated the proton donating ability and could serve as free radical inhibitor or scavenger. In fact, the radical scavenging capability of phenolic compounds are due to their hydrogen donating ability/number of hydroxyl groups present, which in turn, is closely related both to the chemical structure and spatial conformation, that can modify the reactivity of the molecules (28).

Table 3. Effects of the EA extract of *Polyalthia simiarum* on hematological parameter in EAC bearing mice.

Treatment	RBC (cell × 10 ³ /mm ³)	WBC (cell × 10 ³ /mm ³)	Hemoglobin (g %)
Normal control	5.39 ± 0.12	3.92 ± 0.32	13.90 ± 3.1
EAC control	3.91 ± 0.80*	5.84 ± 0.52*	4.95 ± 1.80*
EA extract (50mg/kg)	4.83 ± 0.66**	4.89 ± 0.32**	7.93 ± 1.62**
EA extract (25mg/kg)	3.02±0.03**	3.03±0.28**	4.25±0.144**
Bleomycin (0.3 mg/kg)	5.18 ± 0.12**	3.15 ± 0.83**	12.89 ± 2.93**

Each point represent the mean ± SEM. (n=6 mice per group), **p*<0.001 statistically significant when compared with control group, ***p*<0.005 statistically significant when compared with EAC control group.

In EAC-treated tumor bearing mice, a regular rapid increase in ascetic tumor volume was observed. Ascetic fluid is the direct nutritional source for tumor cells and a rapid increase in ascetic fluid with tumor growth would be a way to meet the nutritional requirement of tumor cells (29). Treatment with EA extract of *P. simiarum* reduced the intraperitoneal tumor burden, thereby reducing the tumor volume, tumor weight, viable tumor cell count and increased the life span of the tumor bearing mice. The steadfast criteria for judging the potency of any anticancer drug are prolongation of life span of animals (30). It can therefore be inferred that the increase in life span of EA extract treated EAC bearing mice may be due to the decrease in the nutritional fluid volume and delay the cell division (31).

Reduction in viable cell count and increased non viable cell count towards normal in tumor host suggest antitumor effect against EAC cell in mice. These demonstrated that the EA extract have direct relationship with tumor cells as these tumor cells are absorbed the anticancer drug by direct absorption in peritoneal cavity and this anticancer agent promote lysis of the cells by direct cytotoxic mechanism (32). Anemia and myelosuppression have been frequently observed in ascites carcinoma (33). Anemia is encountered in ascites carcinoma mainly due to iron deficiency, either by haemolytic or myelopathic conditions which finally lead to reduced RBC number (34). Treatment with EA extract brought back the hemoglobin level, RBC and WBC count more or less to normal levels, thus supporting its haematopoietic protective activity without inducing myelotoxicity, the most common side effects of cancer chemotherapy.

Preliminary phytochemical studies indicated the presence of alkaloid, steroid, phenolic and flavonoid compounds in EA extract of *P. simiarum*. Literature survey shows that most of the polar compound i.e., alkaloids, flavonoids, phenolics etc are biologically active. Less polar compounds like simple alkaloids, flavonoids, terpenes and their oxygenated derivatives as well as more functional group containing compounds would be successfully extracted with ethyl acetate. Rana *et al.* (35) agreed with the present study. A number of scientific reports indicated that certain terpenoid, steroid and phenolic compounds and flavonoids have chemo preventive role in cancer through their effects on signal transduction in cell proliferation and angiogenesis (36). Phytosterols are able to be incorporated into the cell membrane, alter membrane fluidity and the activity of membrane bound enzymes. They alter signal transduction pathways leading to tumor growth and stimulate apoptosis in tumor cell lines. They have also shown to enhance *in vitro* human peripheral blood lymphocyte and T-cell proliferation *in vitro*, which suggests a possible stimulation of the immune system (37). Furthermore, flavonoids such as quercetin, kaemferol and their glycosides have been shown to possess antimutagenic and antimalignant effects (38). The antitumor activities of EA extract of *P. simiarum* are probably due to one or more of alkaloids, phenolic compounds, flavonoids as well as terpenoids present in the extract.

In present study, it was observed that the EA extract of *P. simiarum* exhibited antioxidant activity as well as significantly reduced tumor growth, viability of tumor cells, normalized the hematological profiles and increased life span as compared with those of EAC treated control mice. Further chemical studies are underway to isolate the bioactive compounds responsible for these bioactivities.

ACKNOWLEDGEMENT

The authors are thankful to the Department of Applied Chemistry & Chemical Engineering, University of Rajshahi, Rajshahi, Bangladesh, for providing laboratory facilities to carry out this research work.

REFERENCES

1. Dashora N, Sodde V, Bhagat J, Prabhu KS, Lobo R, Antitumor activity of dendrophthoe falcate against Ehrlich ascites Carcinoma in swiss albino mice, *Pharm Crops* 2, 1-7, 2010.
2. Rajkumar V, Guha G, Kumar , Antioxidant and anti-neoplastic activities of *Picrorhiza kurroa* extracts, *Food Chem Toxicol* 49, 363-369, 2011.
3. Temple NJ, Antioxidants and disease: more questions than answers, *Nutr Res* 20, 449-459, 2000.
4. Suja KP, Jayalekshmy A, Arumughan, Free radical scavenging behavior of antioxidant compounds of sesame (*Sesamum indicum* L.) in DPPH center dot system, *J Agric Food Chem* 52, 912-915, 2004.
5. Sun J, Chu YF, Wu X, Liu RH, Antioxidant and antiproliferative activities of common fruits, *J Agric Food Chem* 50, 7449-7454, 2002.
6. Zhang Y, Seeram NP, Lee R, Feng, L, Heber D, Isolation and identification of strawberry phenolics with antioxidant and human cancer cell antiproliferative properties, *J Agric Food Chem* 56, 670-675, 2008.
7. Meyskens FL, Szabo ,. Diet and cancer: The disconnect between epidemiology and randomized clinical trials, *Cancer Epid Biomar* 14, 1366-1369, 2005.
8. Koduru S, Grierson DS, Venter MV, *In vitro* antitumor activity of *Solanum aculeastrum* berries on three carcinoma cells, *Int J Cancer Res* 2, 397-402, 2006.
9. Hafidh RR, Abas F, Abdulamir AS, Jahanshiri F, Abu Bakar F, Sekawi Z, A review: Cancer research on natural products in Asia, *Int J Cancer Res* 5, 69-82, 2009.
10. Kumar RS, Jayakar B, Rajkapoor B, Antitumor activities of *Indigofera trita* on Ehrlich ascites carcinoma induced mice, *Int J Cancer Res* 3, 180-185, 2007.
11. Choi HY, Jhun EJ, Lim BO, Chung IM, Kyung SH, Park DK, Application of flow injection-chemiluminescence to the study of radical scavenging activity in plants, *Phytother Res* 14, 250-253, 2000.
12. Kirtikar KR, Basu BD, *Indian Medicinal Plants*, Singh B, Singh MP (Eds), Dehra Dun: India, 1980.
13. Rashid MA, Hossain MA, Hasan CM, Reza, MS, Antimicrobial diterpenes from *Polyalthia longifolia* var *pendulla* (Annonaceae), *Phytother Res* 40, 79-81, 1996.
14. Sashidhara KV, Singh SP, Sarker J, Sinha S, Cytotoxic clerodane diterpenoids from the leaves of *Polyalthia longifolia*, *Nat Prod Res* 24, 1687-1694, 2010.
15. Kanokmedhakul S, Kanokmedhakul K, Yodbuddee D, New antimalarial bis-dehydroaporphine alkaloids from *Polyalthia debilis*, *J Nat Prod* 6, 616-619, 2003.
16. Li HY, Sun NJ, Kashivada Y, Sun L, Snider JV, Cosentino LM, Lee KH, Anti-AIDS agents, 9. Suberosol, a new C31 lanostane-type triterpene and anti-HIV principle from *Polyalthia suberosa*, *J Nat Prod* 56, 1130-1133, 1993.
17. Kabir S, Chowdhury AMS, Rashid MA, Antimicrobial and cytotoxic activities of the crude extracts of *Polyalthia simiarum*, *Dhaka Univ J Sci* 59,199-200, 2011.
18. <http://www.mpbd.info/plants/polyalthia-longifolia.php>.
19. Kabir S, Rahman MS, Chowdhury AM, Hasan CM, Rashid MA, An unusual bisnor-clerodane diterpenoid from *Polyalthia simiarum*, *Nat Prod Commun* 5, 1543-1546, 2010.
20. Yarnalkar S, *Practical Pharmacognosy*, Nirali Prakashan Pune, Maharashtra, 1991.
21. Lorke DA, A new approach to practical acute toxicity testing, *Arch Toxicol* 54, 275-287, 1983.
22. Braca A, Tommasi ND, Bari LD, Pizza C, Politi M, Morelli I, Antioxidant principles from *Bauhinia terapotensis*, *J Nat Prod* 64, 892-895, 2001.
23. Rana AYKMM, Khanam JA, *Aristolochia indica* whole plant extract as an antineoplastic agent, *J Med Sci* 2, 202-205, 2002.
24. Sur P, Ganguly DK, Tea plant roots extract (TRE) as an antineoplastic agent, *Planta Med* 60, 106-109, 1994.

25. Armour DFE, Blood FR, Belden DA, The Manual for Laboratory Works in Mammalian Physiology, University of Chicago Press: United State, 1965.
26. Nakayama T, Suppression of hydroperoxide-induced cytotoxicity by polyphenols, Cancer Res 54, 1991-1993, 1994.
27. Chang CC, Yang MH, Wen HM, Chern JC, Estimation of total flavonoid content in propolis by two complementary colorimetric methods, J Food Drug Anal 10, 178-182, 2002.
28. Gorelik S, Legumsky M, Kohen R, Joseph KJ, A novel function of red wine polyphenols in humans: Prevention of absorption of cytotoxic lipid peroxidation products, The FASEB J 22, 41-46, 2008.
29. Prasad SB, Giri A, Antitumor effect of cisplatin against murine ascites Dalton's lymphoma, Indian J Exp Biol 32, 155-162, 1994.
30. Clarkson BD, Burchenal JH. Preliminary screening of antineoplastic drugs, Prog Clin Cancer 1, 625-629, 1965.
31. Sur P, Bag SP, Khanam JA, Choroacetohydroxamic acid as antitumor agent against Ehrlich ascites carcinoma in mice, Neoplasma 44, 197-201, 1997.
32. Kennedy DO, Kokima A, Hasuma T, Yano Y, Otani S, Matsui-Yuasa I, Growth inhibitory effect of green tea extract and (-)-epigallocatechin in Ehrlich ascites tumor cells involves a cellular thiol-dependent activation of mitogenic activated protein kinase, Chem Biol Interact 134, 113-133, 2001.
33. Hogland HC, Hematological complications of cancer chemotherapy, Semin Oncol 9, 95-102, 1982.
34. Gupta M, Mazumder UK, Halder PK, Chandi CK, Manikandan L, Senthil, GP, Anticancer activity of *Indigofera aspalathoides* and *Wedelia calendulaceae* in swiss albino mice, Iran J Pharm Res 6, 141-145, 2007.
35. Rana AYKMM, Khanam JA, Asad-Ud-Daula, M, Antineoplastic screening of some medicinal plants against Erlich Ascites carcinoma in mice, J Med Sci 4, 142-145, 2004.
36. Blois MS, Antioxidant determination by the use of a stable free radical, Nature 26, 1199-1200, 2002.
37. Jones PJ, Abumweis SS, Phytosterols as functional food ingredients: Linkages to cardiovascular disease and cancer, Curr Opin Clin Nutr Metab Care 12, 147-151, 2009.
38. Fotsis T, Pepper MS., Aktas E, Breit S, Rasku S, Adlercreutz H, Wahala K, Montesano R, Schweigerer L, Flavonoid, dietary-derived inhibitors of cell proliferation and *in vivo* angiogenesis, Cancer Res 57, 2916-2921, 1997.

Received: 24.11.2011

Accepted: 31.10.2012