ClinicSearch

Clinical Oncology Case Reports

Sadikuj Jaman *

Open Access

Research Article

Assessment of Antioxidant and Antineoplastic Activities Blumea Lacera (burn. F) Leaves.

Rokibul Islam Bhuiyan, Sadikuj Jaman*

Department of Biochemistry and Molecular Biology, University of Rajshahi.

***Corresponding Author:** Sadikuj Jaman, Department of Biochemistry and Molecular Biology, University of Rajshahi.

Received date: September 07, 2022; Accepted date: September 13, 2022; Published date: September 20, 2022.

Citation: Rokibul Islam Bhuiyan, Sadikuj Jaman. (2022). Assessment of antioxidant and antineoplastic activities blumea lacera (burn. F) leaves., J. Clinical Oncology Case Reports, 1(1) DOI: 10.31579/2834-5061/004

Abstract:

Blumea lacera (Burn. f.) DC. (Family: Asteraceae) is an important member of Bangladeshi natural plant resource and it is an herbaceous weed locally known as Kukursunga. Different parts of this plant are used traditionally to cure various diseases. But detail study on the antioxidant and antineoplastic potentials of *Blumea lacera* leaves, has not yet been done. Aim of this study is to examine the antioxidant and antineoplastic properties and quantify the different type of phytochemical content of the methanolic extract of leaves of *Blumea lacera* (MELB). MELB contained a rich **polyphenol, flavonol, flavonoid and** proanthocyanidins. MELB showed moderate cytotoxic effect against *Artemia salina* (brine shrimp nauplii) where its LD50 values was 66.12 µg/ml. *In vitro* antioxidant assay, MELB exhibited a remarkable capacity to scavenge the tested reactive species. MELB scavenged DPPH with an IC50 of 33.64 µg/mL and 42.69 µg/mL, respectively. *In vivo* antineoplastic assay, MELB significantly ($P<0.05$) decreased viable cell count and increased the survival time of EAC cell bearing mice. Hematological profiles were also restored significantly ($P<0.05$) to normal levels in MELB treated mice as compared to untreated EAC control mice. In addition, fluorescence microscopic view of EAC cells derived from MELB-treated group showed apoptotic characteristics in treated cells compared to untreated EAC control. our findings suggest that methanolic extract of *Blumea lacera* leaves(MELB) might be a potential agent with antioxidant properties for prevention of cancer and has the merit for further investigation in isolating its active constituents.

Keywords: anti-oxidant; anti-cancer; dpph scavenging activity; melb

Abbreviation:

DPPH: 1,1-diphenyl-2-picrylhydrazyl, **MELB:** methanolic extract of leaves of *Blumea lacera* . **EAC:** Ehrlich's Ascites Carcinoma, **DAPI:** 4,6-diamidino-2-phenylindole

Introduction

Medicinal plants may be defined as a group of plants that possess some special properties or virtues that qualify them as articles of drugs and therapeutic agents and are used for medicinal purposes [1]. Traditional medicinal plants play an important role in modern medicine due to development of research and scientific knowledge. About 25% of the prescription drugs available in markets contain at least one active ingredient derived from plant material [2]. Medicinal uses of plants have been documented in approximately 10,000 to 15,000 of world's plants and roughly 150-200 have been incorporated in western medicine [3]. A good number of secondary metabolites from plants possess interesting biological activities with various applications, such as pharmaceutical ingredients, insecticides, dyes, flavors, and fragrances [3, 4].

The Arabian Muslim physicians, like Al-Razi and Ibn Sina (9th to 12th century AD), brought about a revolution in the history of medicine by bringing new drugs of plant and mineral origin into general use [5].

Population rise, inadequate supply of drugs, prohibitive cost of treatments, side effects of several synthetic drugs and development of resistance to currently used drugs for infectious diseases have led to increased emphasis on the use of plant materials as a source of medicines for a wide variety of human ailments. Recently, WHO (World Health Organization) estimated that 80 percent of people worldwide rely on herbal medicines for some aspect of their primary health care needs [6]. According to them, around 21,000 plant species have the potential for being used as medicinal plants. Worldwide, 50,000–80,000 flowering plants are used for medicinal and therapeutic purposes [7]. Herbalism (also known as herbal medicine or phototherapy) is the study and use of plants intended for medicinal purposes [8]. Globally phytomedicine has been the matter of interest in primary source of healthcare [9] that encouraged its utilization as a source of chemical diversity in drug development [10]. Plants contain enormous number of

natural compounds with important pharmacological properties, and these natural molecules have revolutionized the medicinal system [11, 12].

Recently, all over the world, there is an upsurge and interest among scientific institutions, biological research institutions in the use of medicinal plants, crude extracts or active ingredients to treat various ailments [13-14]. Most of the research that is done on medicinal plants continues to focus on identifying and isolating active ingredients. [15] Compounds which have been identified and extracted from terrestrial plants for their anticancer properties include polyphenols, brassinosteroids and taxols. Plant-derived drugs are desired for anticancer treatment as they are natural and readily available. They can be readily administered orally as part of patient's dietary intake. Also, being naturally derived compounds from plants they are generally more tolerated and non-toxic to normal human cells.

Material and methods

1. Collection and proper identification of the plant Sample

The plant samples selected for this investigation were collected from the area of Rajshahi University campus in August 2018. The plants were authenticated by the authority of Herbarium, Department of Botany, Rajshahi University, Rajshahi where a voucher specimen No. 01 was deposited for *Blumea lacera* (Burn. f.) Drying and pulverization of plant materials, Extraction of powdered plant materials with methanol at room temperature

2. Quantitative analysis of phytochemical compounds

Determination of total phenolic content: The content of total phenolics of methanolic extract of leaves of *Blumea lacera* (Burn. f.) was determined employing the method as described by Singleton et al., (1965) using Folin-ciocalteu reagent (FCR) as oxidizing agent and Gallic acid as standard [16]. Determination of total flavonoid: The content of total flavonoids of *Blumea lacera* (Burn. f.) was determined by Aluminum chloride colorimetric method [17]. Determination of total proanthocyanidins: Proanthocyanidins in the plant extracts were estimated using the previously described method [18].Determination of total flavonols content: Total flavonols in the plant extracts were estimated using the method of Kumaran and Karunakaran(2007). Total content of flavonols was expressed as mg of quercetin equivalent/g of dry extract.

3. Preparation of the test samples

Crude extract such as methanol extract of leaves of *Blumea lacera (*Burn. f.) (MELB) was selected. Gallic acid was used as positive control. All the extracts and gallic acid were measured accurately (10 mg each) in vials and were dissolved in 1% DMSO with 10 ml of distilled water to get a concentration of 10µg/10µl for each of the samples. These solutions were used as stock solution.

4. Experimental animal

Male and female Swiss albino mice (25-30 g) were collected from the Animal Research Branch of Pharmacy department, Jahangirnagar University, Dhaka. The mice were grouped and housed in iron cages with ten animals per cage and maintained under standard laboratory conditions (temperature 25 ± 2 oC; humidity 55 ± 5 %) with 12 hrs dark/light cycle. The mice were allowed free access to standard dry pellet diet (Collected from ICDDR, B) and water *ad libitum*. mice were acclimatized to laboratory conditions for 10 days before beginning of the experiment. The experiments were carried out after approval of the protocol by the Institutional Ethics Committee for Experimentations on animal, human, microbes and living natural sources (225/320-IAMEBBC/IBSc), Institute of Biological Sciences, University of Rajshahi, Bangladesh.

5. Experimental tumor model

Transplantable tumor (Ehrlich's ascites carcinoma) used in this study were obtained from the Department of Pharmacy, Jahangirnagar University, Dhaka and were maintained in our laboratory in Swiss Albino mice by intra peritoneal transplantation.

6. Collection of EAC cells

The EAC cells collected from donor mice (Swiss albino) of 20-27 g body weight were suspended in sterile isotonic saline. A fixed number of viable cells (usually 1.5×106 cells/ml) were implanted into the peritoneal cavity of each recipient mouse, as described in the above experimental method as control. Mice were sacrificed on the sixth day and the intraperitoneal tumor cells were harvested by normal saline.

7. Determination of DPPH• scavenging activity

Phenolic compounds, flavonoids, flavonols and proanthocyanidins function as antioxidants and scavengers of free radicals by rapid donation of a hydrogen atom to radicals. DPPH• is a free radical compound and it has been widely used to test the free radical scavenging ability of antioxidants. Phenolic, flavonoids, flavonols and proanthocyanidins have ability to scavenge 1,1-

diphenyl-2-picrylhydrazyl (DPPH•) free radicals. DPPH free radical is reduced to the corresponding hydrazine when it reacts with hydrogen donors. DPPH can generate stable free radicals in aqueous or methanol solution. With this method, it is possible to determine the anti- radical power of an antioxidant by measuring the decrease in the absorbance of DPPH at 517 nm. In the radical form, this molecule has an absorbance at 517 nm which disappears after acceptance of an electron or hydrogen radical from an antioxidant compound to become a stable diamagnetic molecule.

8. Counting of cells using haemocytometer

The simplest, most convenient and cheapest means of accurately determining the numbers of cells in a sample is to use a haemocytometer and a microscope. A haemocytometer is a specialized slide that has a counting chamber with a known volume of liquid.

9. Measurement of hemoglobin (Hgb)[23]

The amount of haemoglobin (Hgb) was measured by using Shali'shaemometer. 20 µl non coagulating blood was transferred to the cuvette (tube) in haemometer containing a little amount of N/10 HCl. Distilled water was added and stirred until a good color match was obtained. The final reading of the solution in the cuvette was noted. From the cuvette reading gram % (g/dL) of hemoglobin was calculated.

10. Total count of WBC[24]

Exactly 10 µl non coagulating blood was diluted with 1 ml WBC counting fluid and mixed properly. The resultant mixture was checked in haemocytometer and the number of cells was counted with a Microscope. Here the dilution factor was 100. Total WBC cells per ml were calculated.

11. Total count of RBC [25]

Exactly 10 µl non coagulating blood was drawn with the tip of a micropipette and diluted to 1000 times with RBC counting fluid. Total RBC was counted with heamocytometer like WBC counting technique.

12. Statistical analysis

All values were expressed as mean ± SD (Standard deviation). Statistical analysis was performed with one way analysis of variance (ANOVA) followed by Dunnett's't' test using SPSS statistical software of 15 version. $P<0.05$ were considered to be statistically significant when compared with control.

Result:

Total phenolic content in *Blumea lacera* (Burn. f.)

MELB was standardized for their total phenolic contents. The calibration curve showed linearity for Gallic acid in the range of 25 – 400 µg/ml, with a correlation coefficient (R2) of 0.995 and equation y = 0.003x + 0.142

where y is absorbance at 725 nm and x is the concentration of gallic acid in µg/ml (figure 1.1). Total phenolic content in *Blumea lacera* (Burn. f.) was calculated using the formula:

$$TPC=(c\times V) \div m$$

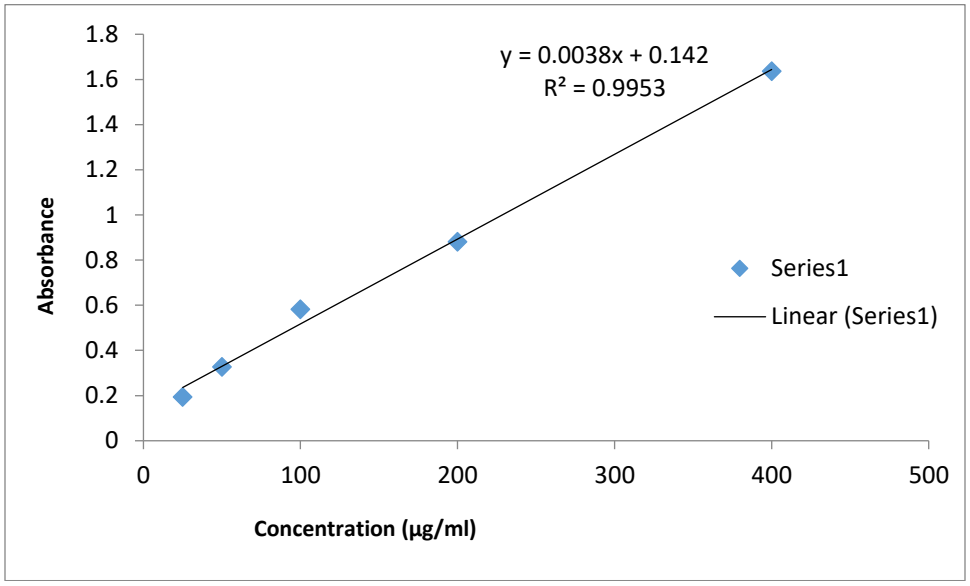

Figure 1: *Standard curve of gallic acid for the determination of total phenolics*

Total flavonoid content in *Blumea lacera* (Burn. f.)

MELB was standardized for their contents of flavonoids compounds. The calibration curve showed linearity for Catechin in the range of 6.13-100µg/ml, with a correlation coefficient (R2) of 0.999 and equation y = 0.009x – 0.004 where y is absorbance at 510 nm and x is the concentration in µg/ml of Catechin (Figure 2.1). Total flavonoids content in *Blumea lacera* (Burn. f.) were calculated using the formula:

$$TFC=(c\times V) \div m$$

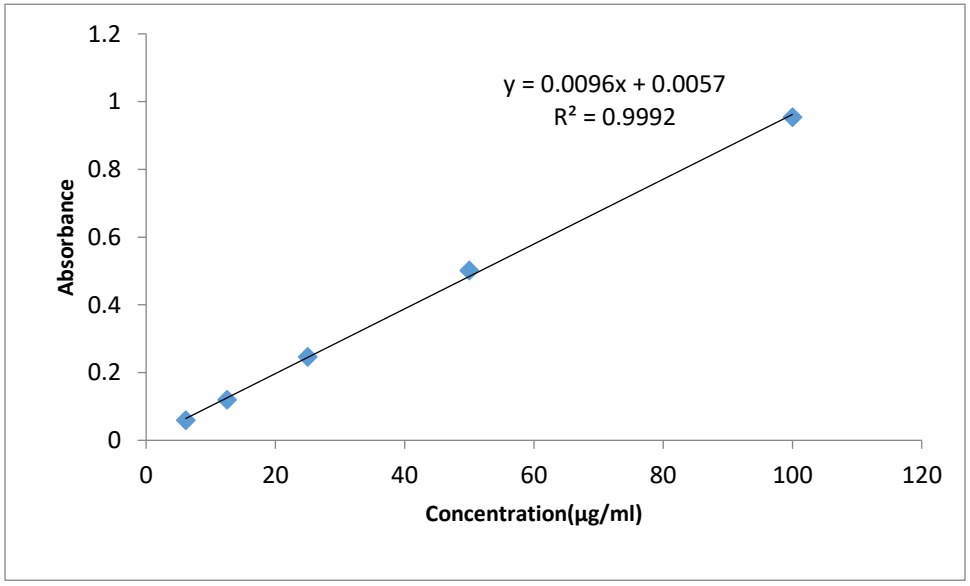

Figure 2: *Standard curve of Catechin for the determination of total flavonoids.*

Total proanthocyanidin contents in *Blumea lacera* (Burn. f.)

Methanol extract of leaves of *Blumea lacera* (Burn. f.) was standardized for their contents of proanthocyanidins compounds. The calibration curve showed linearity for Catechin in the range of 5-100µg/ml, with a correlation coefficient (R2) of 0.995 and equation y = 0.001x + 0.014 where y is absorbance at 510 nm and x is the concentration in µg/ml of Catechin (Figure 3.1). Total proanthocyanidins content in *Blumea lacera* (Burn. f.) were calculated using the formula:

$$TFC=(c\times V) \div m$$

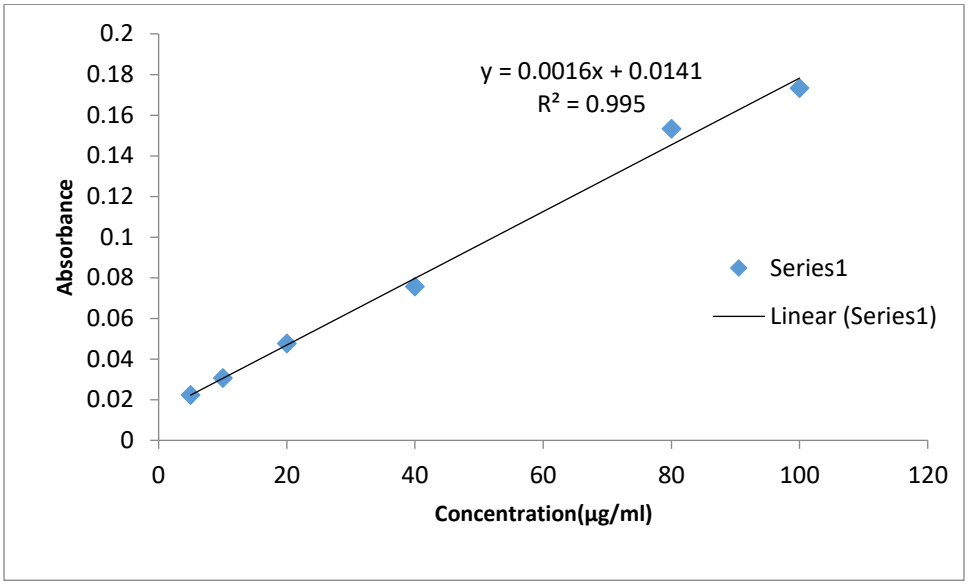

Figure 3: *Standard curve of Catechin for the determination of total proanthocyanins*

Total flavonol contents in *Blumea lacera* (Burn. f.)

Here MELB was standardized for its flavonol contents. The calibration curve showed linearity for quercetin in the range of 50-800µg/ml, with a correlation coefficient (R2) of 0.985 and equation y = 0.002x + 0.090 where y is absorbance at 510 nm and x is the concentration in µg/ml of quercetin (Figure 4.1). Total flavonols content in MELB were calculated using the formula:

$$TFC=(c\times V) \div m$$

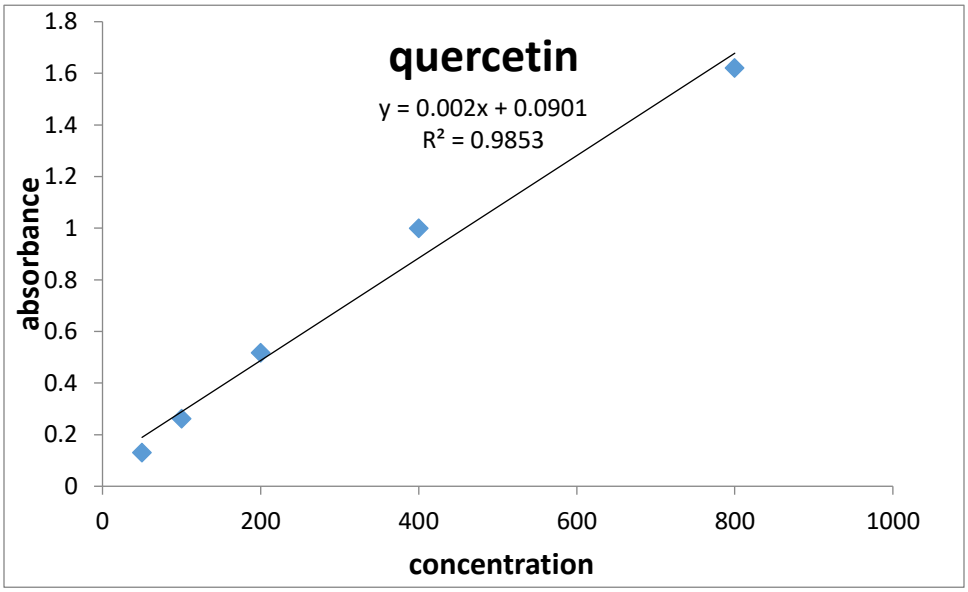

Figure 4: *Standard curve of quercetin for the determination of total flavonols*

DPPH radical scavenging activity

MELB and ascorbic acid showed free radical scavenging activity as shown in figure 5.1. The different concentration of MELB.and standard (Ascorbic acid) exhibited antioxidant activities in a dose dependent manner. The scavenging activity of *Blumea lacera* (Burn. f.) was promising when compared with standard ascorbic acid. The IC50 of *Blumea lacera* (Burn. f.) and ascorbic acid were 33.64 µg/ml and 29.03 µg/ml respectively

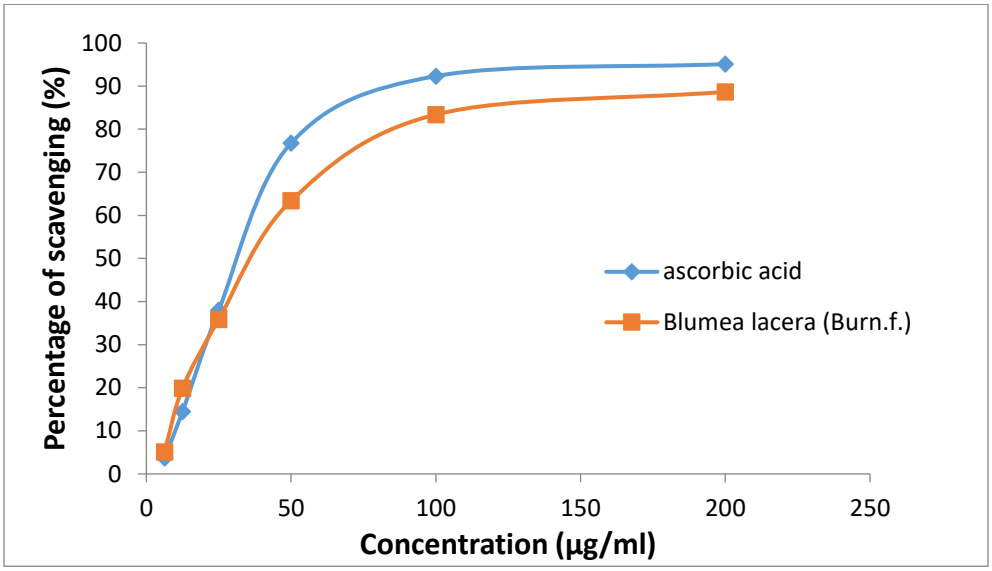

Figure 5: *DPPH radical scavenging activity of Blumea lacera (Burn. f.) And Ascorbic acid as reference standard.*

An overall comparison of IC50 value of *Blumea lacera* (Burn. f.) with Ascorbic acid is given in the following graph:

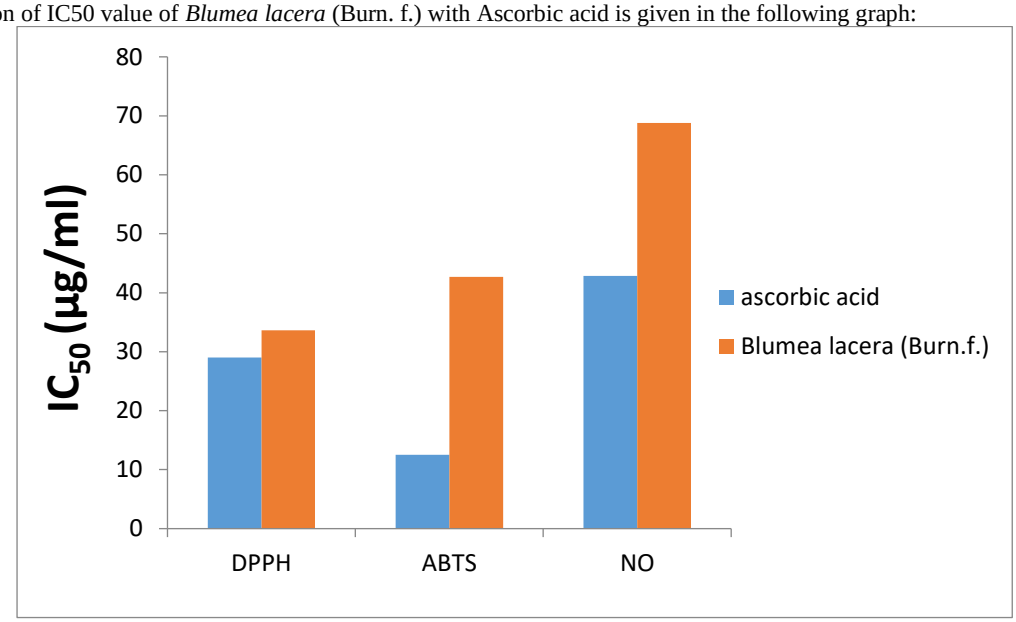

Figure 6: *An overall overview of IC50 value of Ascorbic acid and Blumea lacera (Burn. f.)*

Brine shrimp lethality bioassay:

The methanol extract of leaves of MELB showed positive result indicating that the extract was biologically active. The crude extract of MELB showed moderate cytotoxic activity with LD50 value of 66.125µg/ml in comparison with gallic acid standard whose LD50 value is 7.2 µg/ml (Table 6.1). In this bioassay, the mortality rate of brine shrimp was found to be increased with the increase in concentration of the test sample (Table 6.1). So it was observed that there is a positive correlation between brine shrimp toxicity and cytotoxicity

Test sample	LD50 (µg/ml)	95% Confidence limits (µg/ml)	Regression equation	χ2 value (Degrees of freedom-df)
Gallic acid	7.21±0.47	4.00 to 13.03	y =3.117+2.213x	0.049 (1)
MELB	66.12±0.80	37.5949 to 116.3072	y=1.9859+1.655x	0.39 (3)

Table 6.1: *Concentration dependent cytotoxic potential of crude methanol extracts of MELB and standard against Brine shrimps nauplii.*

***In vivo* antineoplastic activity:**

MELB against EAC cell bearing mice was assessed by the parameters such as viable EAC cell (% inhibition in cell growth), mean survival time (MST), percentage (%) increase in life span (%ILS) and body weight gain. The average number of viable tumor cells per mouse of untreated EAC control group was found to be (6.75 ± 0.56) x 106 cells/ml. Treatment with MELB (25 mg/kg and 50 mg/kg) decreased the viable cells significantly (P<0.05) (Fig 7.1). MELB showed 62.52% and 80.24 % EAC cell growth inhibition at the dose of 25 and 50 mg/kg body weight, respectively (Fig. 7.2). Moreover, DAPI staining showed

reduction in the number of EAC cells and fluorescence microscopic view of EAC cells derived from treated groups demonstrated the alterations (i.e nuclear condensation, fragmentation, membrane blabbing) in the cell morphology of the EAC cells when compared with untreated control (Fig 7.3 to 7.4).

The effect of MELB on the survival of EAC bearing mice is shown in figure 7.5. The MST of the untreated control group was 22.60±1.36days, whereas it was 30.66 ± 1.52and39.67 ±1.15 (P<0.05) for the group treated with MELB at 25 and 50 mg/kg, respectively. The increase in the life span of EAC cell bearing mice treated with MELB (25 and 50mg/kg) was found to be 35.67 % and75.53 % (figure 7.5). On 15th day of EAC cell inoculation, the average weight gain of only EAC cell bearing mice was 28.85 ± 0.92g whereas it was 18.83 ± 1.46 (P<0.05) and 8.84 ± 0.65 (P<0.05) for the groups treated with MELB at 25 and 50 mg/kg, respectively (figure 7.5). All these are considered as important and promising aspects in justifying the potency of a compound in cancer chemotherapy.

Hematological parameters of untreated EAC cell bearing mice on the day 13 were showed significant (P<0.05) changes when compared to normal mice (Table 7.1). The total WBC count was found to increase with a reduction in the hemoglobin content and total RBC count in EAC cell bearing mice. At the same time interval, treatment of EAC cell bearing mice with MELB (25 and 50 mg/kg) restored all the altered hematological parameters toward normal range.

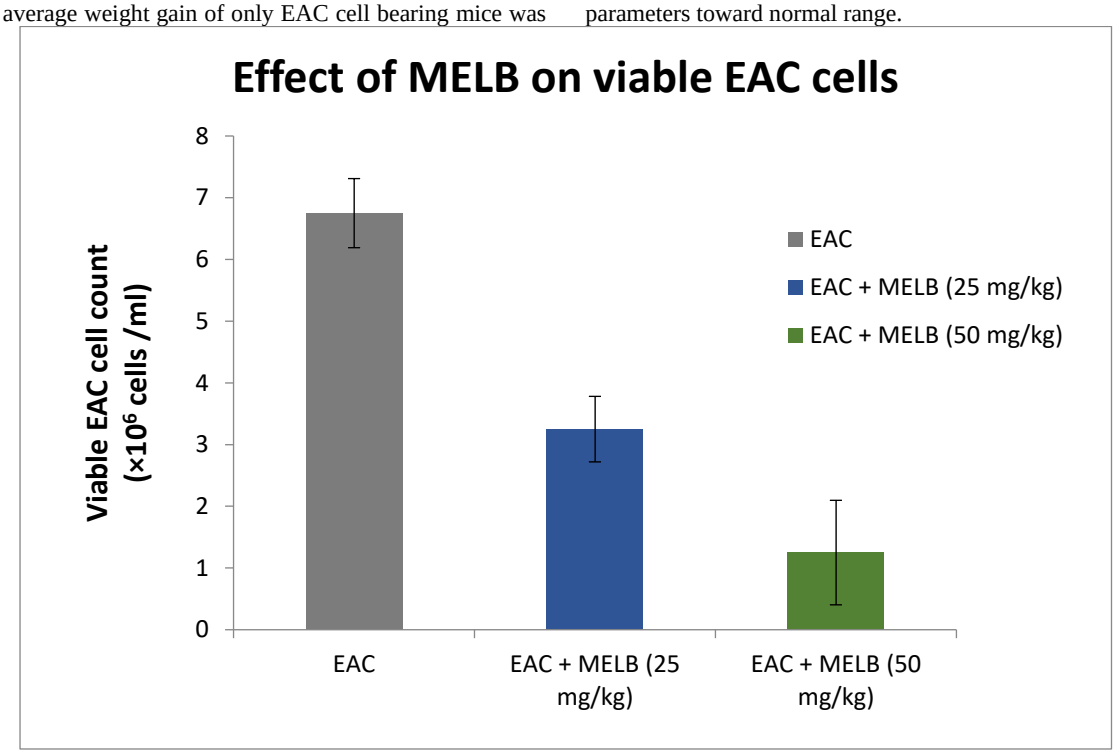

Figure 7.1 : *Effect of MELB on Viable EAC cells on the day 6 after tumor cell inoculation. Data are expressed as mean ± SD (n = 6); *P<0.05: Significance difference with respect to EAC control.*

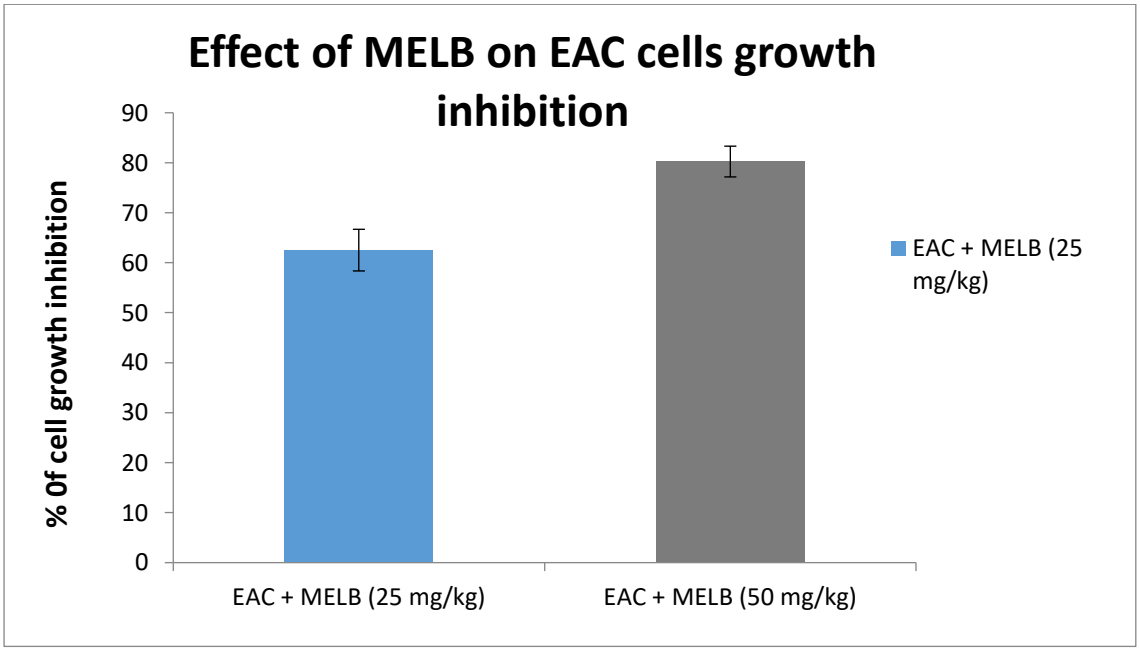

Figure 7.2: *Effect of MELB on % of cell growth inhibition; Data are expressed as mean ± SD (n = 5); *P<0.05: Significance difference with respect to EAC control.*

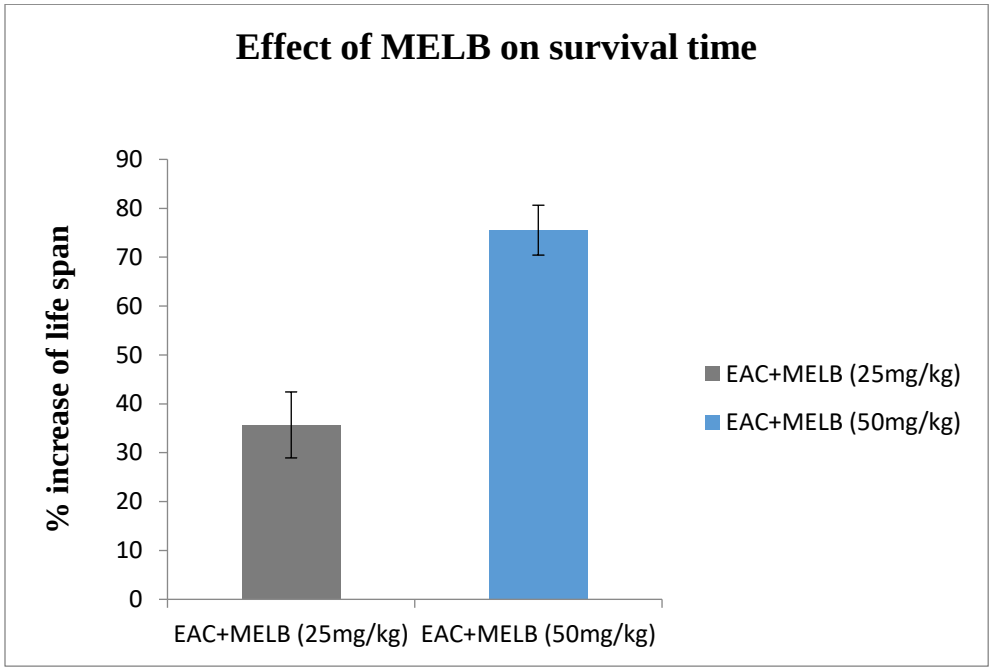

Figure 7.3: *Percentage increase of life span (%ILS) of MELB treated EAC cell bearing mice. Data are expressed as mean ± S.D (n = 8). *P<0.05: Significance difference with respect to EAC control.*

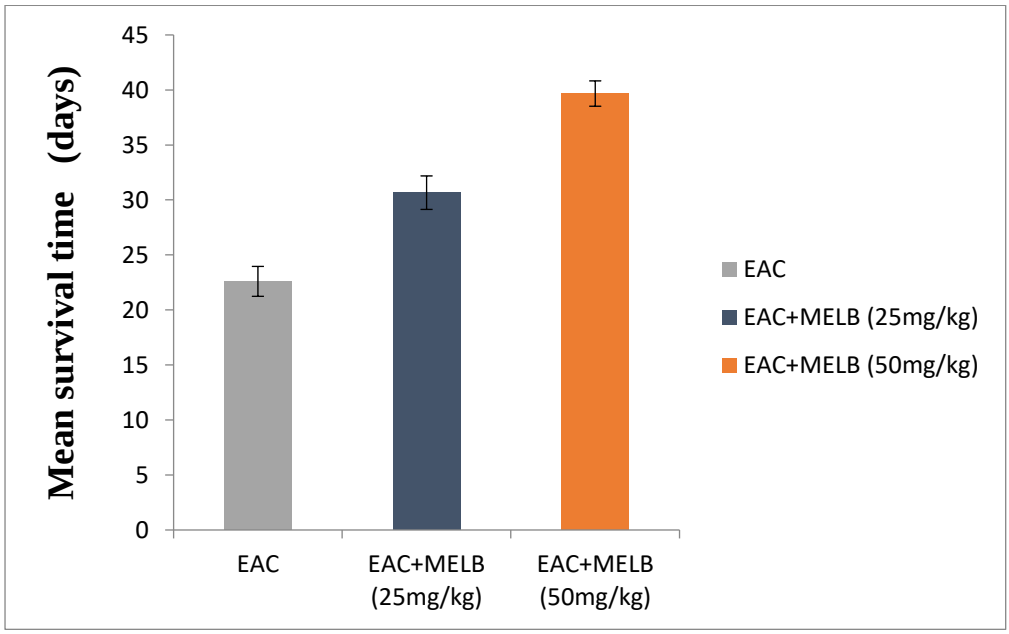

Figure 7.4: *Mean survival time (MST) with MELB treated EAC cell bearing mice. Data are expressed as mean ± S.D (n = 8). *P<0.05: Significance difference with respect to EAC control.*

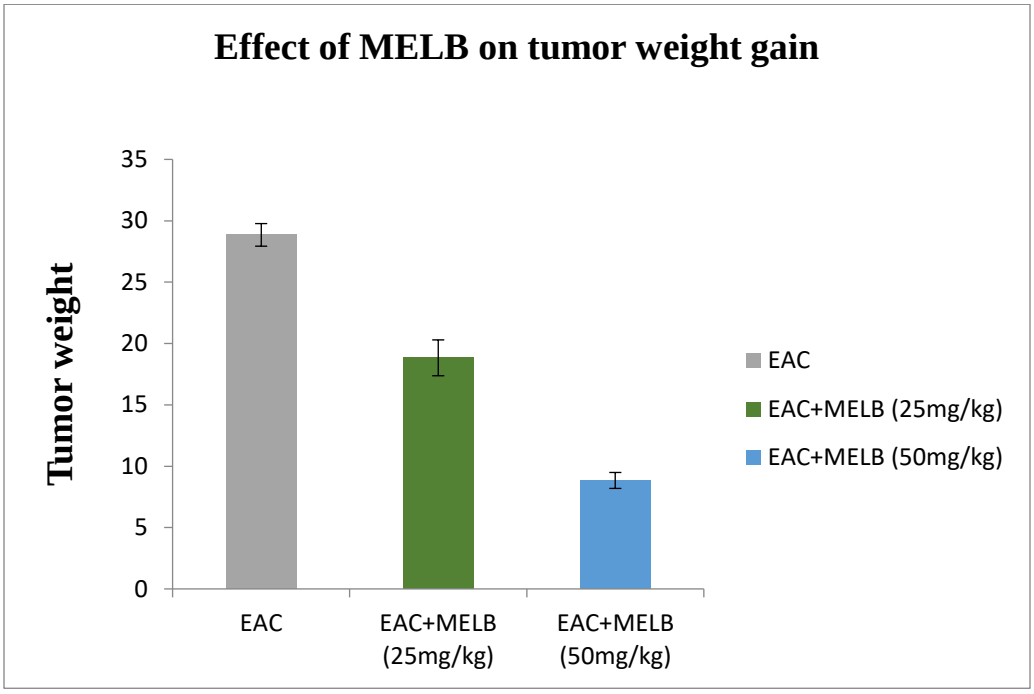

Figure 7.5: *Effect of MELB on tumor weight gain in tumor bearing mice after 14 days. Data are expressed as mean ± S.D (n = 8). *P<0.05: Significance difference with respect to EAC control.*

Parameters	Normal	EAC+control	EAC+MELB (25 mg/kg)	EAC+MELB (50 mg/kg)
Hgb (g/dL)	11±1	6.1 ± 1.10*	7.9 ±1.08t	8.5 ±1.13t
RBC(x107 cells/mL)	3.5±0.07 0.070.07	1.4±0.02* 1.46*	2.02± 0.25t	2.45 ± 0.55t
WBC(x106 cells/ml)	20±0.50	75.5±2.53*	51 ± 2.42t	40.67 ± 2.53t

Data are expressed as mean ± S.D for eight animals in each group. *P<0.05; against normal group and tP<0.05: against EAC control group.

Table 7.1. *Effect of MELB on blood parameters of tumor bearing and normal swiss albino mice.*

Discussion:

Extensive research has been done on the ability of antioxidant compounds to prevent various human cancers. As flavonoids are the most abundant antioxidant found in the plant kingdom, a number of plant species with rich flavonoid contents have been reported to possess high therapeutic efficiency for the treatment of cancer [25]. In addition, phenolic contents of plant kingdom have also produced remarkable effects in the prevention of numerous oxidative stress associated diseases including cancer due to their strong antioxidant activities [26,27]. In this investigation, we found MELB as a rich source of flavonoid and phenolic compounds.

Furthermore, we employed several *in vitro* assay models to assess the antioxidant activity of MELB. As a free radical scavenger, plant extracts or compound donate hydrogen or electron to DPPH and thus the radicals become reduced. This phenomenon has been used in DPPH assays to determine free radical scavenging properties of plant sample [28]. In addition, excessive NO undergoes reaction with oxygen to generate toxic free radicals like nitrite and peroxynitrite anions although NO plays important role in many physiological processes and regulation of cell mediated toxicity [29,30]. This study demonstrated notable antioxidant activity of MELB in DPPH (IC50; 33.64 µg/mL), which suggests its probable use in the treatment of various oxidative stress related diseases including cancer.[31].

EAC cells offer special benefits for anticancer drug test due to their suitability to study in almost any mouse host; hence they were used in the present investigation. Moreover, these cells lack H-2 histocompatibility antigens, which is the probable reason for their quick proliferation. *In vivo* anticancer activity of MELB was evaluated by using EAC cell-bearing mice model and the results showed that untreated EAC cell-bearing mice gained body weight (28.85 ± 0.92 g) rapidly due to tumor burden but the treatment of the EAC-cell bearing mice with MELB reduced body weight gain (18.83 ± 1.46 and 8.84 ± 0.65 g at 25 and 50 mg/kg doses) significantly (P<0.05) (Figure 8.5). MELB significantly (P<0.05) decreased the viable EAC cells of treated group in respect to untreated EAC control thereby producing 62.52% and 80.24 % cell growth inhibition at the dose of 25 and 50 mg/kg body weight, respectively. Mean survival time of MELB-treated groups (30.66 ± 1.52 and 39.67 ±1.15 days at 25 and 50 mg/kg doses) was also increased when compared with untreated control (22.60 ±1.36 days) and the percentage (%) increase of life span was found to be 35.67 and 75.53% at 25 and 50 mg/kg doses, respectively (Figure 7.4).

Reduction of average body weight gain, cell growth inhibition and enhancement of life span of tumor bearing mice are measured for the judgment of potency of a certain compound as anticancer agent. The data obtained from this study are consistent with those reported in the literature [14, 31], suggesting that MELB possesses potent anticancer activity.

Further evaluation of anticancer activity of MELB was carried out by examining the change in hematological parameters. EAC cells bearing mice were found to reduce the hemoglobin contents and RBC counts gradually, the major problems in cancer bearing mice. Administration of MELB in EAC cell-bearing mice reversed back all the altered

hematological parameters more or less to normal level (Table 7.1) and this result is similar with that of previous studies where EAC cell-bearing mice were treated with bioactive compounds [31,32], suggesting the protective action of MELB on the hemopoetic system.

Apoptosis, a cell-suicidal mechanism, is characterized by the change of morphological features, such as nuclear fragmentation, blabbing, cell shrinkage and chromatin condensation [33]. In our present study, when EAC cells were treated with MELB, cell shape was changed and the nucleus was fragmented and condensed. After comparison with the control EAC cells suggested that MELB can play significant role in cancer prevention by inducing apoptosis.[34] Induction of apoptosis in EAC cells by different plant extracts has been revealed by several previous studies [35,36].

Conclusion: We have achieved the following findings from this study: MELB contained rich amount of polyphenolic and flavonoids compounds. MELB had remarkable free radical DPPH scavenging properties and showed moderate cytotoxic effect against *Artemia salina* (Brine Shrimp nauplii).[37] In antineoplastic study, treatment with MELB significantly decreased the viable EAC cells and body weight gain thereby increasing the life span of EAC bearing mice and also brought back the altered hematological parameters to normal values. MELB showed its activity against EAC cells by inducing apoptosis as investigated by observing morphological changes of MELB-treated EAC cells under fluorescence microscope.[38,39, 40]

From the above results it can be concluded that antioxidant and antineoplastic abilities of the methanolic extract of *Blumea lacera* leaves render its suitability to be considered as a source for the development of anticancer drug. [41, 42] However, it is necessary to carry out extensive research on leaves of this plant to isolate the active compounds responsible for antioxidant and antineoplastic activities. In future, our research will be forwarded along this direction.

Acknowledgement: This Research paper was originally experimented and written by Md Rakibul Islam Bhuiyan during thesis study. Then this paper was reviewed and corrected by Md. Sadikuj Jaman. The all cost or funding for experiment was carried out by department of biochemistry and molecular biology, University of Rajshahi, Rajshahi 6205.

References:

1. Medicinal plants and their importance as alternative medicine, posted: 30/12/2011. In food, Health, Natural medicine, Research, Uncategorized.
2. Lewington A. Plants for People. Natural History Museum, London, UK. 1990; p. 135-136.
3. McChesney JD, Venkataraman SK, Henri JT. (2007) Plant natural products: back to the future or into extinction? Phytochemistry. 68(14):2015-22
4. Birari RB, Bhutani KK. (2007) Pancreatic lipase inhibitors from natural sources. Drug Discovery Today. 12:879-889.
5. Fuller HJ, Carothers ZB, (1967) The Plant world, 4th edn, Holt Rinehart and Winston, Inc. p.1-4.
6. Organization, World Health Organization (2002) WHO Traditional Medicine Strategy: 2002-2005 (document WHO/ EDM/TRM/2002.1). Geneva.
7. Marinelli J (2005) Plant: The Ultimate Visual Reference to Plants and Flowers of the World. New York, NY: DK Publishing Inc.; 200.
8. Ashraf MA, Khatun A, Sharmin T, Mobin F, Tanu AR, Morshed T, Fakir TA et. Al, (2014) MPDB 1.0: a medicinal plant database of Bangladesh. Bioinformation. 10(6):384-386
9. Farnsworth,N.R. and Morris,R.W. (1976) Higher plants—sleeping giant of drug development. Am. J. Pharm. Sci. Support. Public Health. 148, 46–52.
10. .Evans,B.E., Rittle,K.E., Bock,M.G. et al. (1988) Methods for drug discovery: development of potent, selective, orally effective cholecystokinin antagonists. J. Med. Chem.31, 2235–2246.
11. Mishra,B.B. and Tiwari,V.K. (2011) Natural products: an evolving role in future drug discovery. Eur. J. Pharmacol. Med. Chem.46, 4769–4807.
12. Koehn,F.E. and Carter,G.T. (2005) The evolving role of natural products in drug discovery. Nat. Rev. Drug Discov. 4, 206–220
13. Wakdikar S. (2004) Global health care challenge: Indian experiences and new prescriptions. Electronic Journal of Biotechonology. 7:217-223.
14. Hoareau L, Dasilva EJ. (1999) Medicinal plantsL a reemerging health aid. Electronic Journal of Biotechonology. 2:56-70.
15. Importance of medicine, Stevia, The sweet herb, December 29, 2005.
16. Md sadikujjaman, md abu Sayeed, (2018) "Ellagic Acid, sulphoraphan, and ursolic acid in the prevention and therapy of breast cancer: current evidence and future perspectives" Breast Cancer 25: 517.
17. Noronha V, Tsomo U, Jamshed A, Hai M, Wattegama S, et al. (2012) fresh look at oncology facts on south central Asia and SAARC countries.South Asian J Cancer.; 1(1):1-4.
18. SMA Hussain, (2013) Comprehensive update on cancer scenario of Bangladesh. South Asian J Cancer. 2(4): 279–284.
19. Jaman MS, Rahaman MS, swarma RR, Mahato J et al. (2018) diabetes and red blood cell parameters Ann Clin Endocrinol Metabol.2018; 2: 001-009.
20. Saha MN, Alam MA, Aktar R, Jahangir R, (2008) In vitro free radical scavenging activity of Ixora coccinea L. Bangladesh J Pharmacol 3:90-96.
21. Hazra B, Santana B, Nripendranath M, (2008) Antioxidant and free radicals scavenging activity of Spondias pinnata. BMC Complement Altern Med 8:63.
22. Oyedemi SO, Bradley G, Afolayan AJ, (2010) In vitro and In vivo antioxidant activities of aqueous extract of Strychonos henningsii Gilg. Afr J Pharm Pharmacol 4:70-78.
23. Raghuveer C, Tandon RV, (2009) Consumption of functional food and our health concerns. Pak J Physiol 5(1): 76-83.
24. Hosseinimehr SJ, Pourmorad F, Shahabimajd N, (2007) In vitro antioxidant activity of Polygonium lyrcanicum, Centaureae depressa, Sambus ebulus, Menthe spicata and Phytolaceae Americana. Pak J Biol Sci 10(4): 637-640.
25. Robak J, Marcinkiewicz E (1995) Scavenging of reactive oxygen species as the mechanism of drug action. Pol J Pharmacol Pharm 47:89-98.
26. Mathew S, Abraham TE (2006) In vitro antioxidant activity and scavenging effects of Cinnamomum verum leaf extract assayed by different methodologies. Food Chem Toxicol 44(2): 198-206.
27. Jaman S, Rezwan S, Alam S, Islam R, Husna A U, Sayeed S (2017) association of mean platelet volume and platelet distribution width with HbA1c Journal of Endocrinology and Diabetes, 4(4): 1-6
28. Madhavi DL, Salunkhe DK (1995) Toxicological aspects of food antioxidants. In: Madhavi DL, Deshpande SS, Salunkhe DK, editors. Food antioxidants. New York: Dekker;. p. 267.
29. Veerapur VP, Prabhakar KR, Parihar VP, Kandadi MR, Ramakrishana S, Mishra B, et al, (2009) Ficus racemosa stem bark extract: A potent antioxidant and a probable natural radioprotector. Evid Based Complement Alternate Med 6:317-324.
30. Choi HY, Jhun EJ, Lim BO (2000) Application of flow injection-chemilumineacence to the study of radical scavenging activity in plants.Phytother Res, 14: 250-253.