OPEN ACCESS Freely available online
www.banglajol.info/index.php/JSF
Journal of Science Foundation
July 2020, Vol. 18, No. 2, pp. 72-80
ISSN (Print) 1728-7855
DOI: https://doi.org/10.3329/jsf.v18i2.52781

Original Article

Evaluation of Antioxidant, Antitumor, Analgesic and Anti-inflammatory Activities of *Glochidion lanceolarium* (Roxb.) Voigt

Selina Kabir[1], Ronok Zahan[2], Abdullah Mohammad Sarwaruddin Chowdhury[3], Mohammad Abdur Rashid[4], Mohammad Rashedul Haque[5], Choudhury Mahmood Hasan[6]

Abstract

The present study was designed to investigate the antioxidant, antitumor, analgesic and antiinflammatory activities of methanol extract (MEGL) and petroleum ether fraction (PEFGL) and carbontetrachloride fraction (CTFGL) of stem bark of *Glochidion lanceolarium* (Euphorbiaceae). Antioxidant potential was evaluated in vitro by DPPH (1,1diphenyl-2-picrylhydrazyl) scavenging assay method. The MEGL, PEFGL and CTFGL showed free radical scavenging activity with IC50 value of 18.32 ± 0.76, 20.29 ± 0.79 and 20.24 ± 2.02 µg/ml, respectively. The antitumor activity of the extract/fractions were determined against Ehrlich ascites carcinoma (EAC) in mice at 20 mg/kg body weight intraperitoneally. Significant ($p<0.001$) increase of survival time by 25 ± 0.32, 26 ± 0.10 and 23 ± 0.19 days by the MEGL, PEFGL and CTFGL treated tumor bearing mice was confirmed with respect to the control group (20 ± 0.12 days). Hematological studies revealed that the heamoglobin (Hb) content was decreased in EAC treated mice whereas restoration to close to normal levels was observed in extract/fractions treated animals. Both extract and fractions at the dose of 100 mg/kg b.w., produced significant increase in pain threshold in tail immersion method whereas significantly reduced the writhing caused by acetic acid induced method. The MEGL, PEFGL and CTFGL showed anti-inflammatory activities at the dose 100 mg/kg body weight. Among all the extract/fractions the CTFGL showed comparable best analgesic activity in all the tested methods and also reduced the paw edema (0.85±0.07 after 4h) when compared to carrageenan induced control mice. Therefore, the MEGL, PEFGL and CTFGL of *G. lanceolarium* was capable to exhibit moderate antioxidant, antitumor analgesic and anti-inflammatory activities. This is the first report of antioxidant, antitumor, analgesic and anti-inflammatoryr potential of *G. lanceolarium*. [*Journal of Science Foundation, January 2020;18(2):72-80*]

Keywords: *Glochidion lanceolarium*; DPPH; antitumor; analgesic; anti-inflammatory

*[**Reviewed:** 3 March 2020; **Accepted on:** 1 April 2020; **Published on:** 1 July 2020]*

[1]Department of Applied Chemistry & Chemical Engineering, University of Dhaka, Bangladesh
[2]Department of Pharmacy, Rajshahi University, Bangladesh
[3]Department of Applied Chemistry & Chemical Engineering, University of Dhaka, Bangladesh
[4]Department of Pharmaceutical Chemistry, University of Dhaka, Bangladesh
[5]Department of Pharmaceutical Chemistry, University of Dhaka, Bangladesh
[6]Department of Pharmaceutical Chemistry, University of Dhaka, Bangladesh

Correspondence: Selina Kabir, Department of Applied Chemistry & Chemical Engineering, University of Dhaka, Bangladesh; **Email:** selinakabir.bd@gmail.com

Introduction

Free radicals cause depletion of immune system, antioxidants change in gene expression and induce abnormal proteins and contribute to more than one hundred disorders in humans including atherosclerosis, arthritis, ischemia and reperfusion injury of many tissues, central nervous system injury, gastritis, diabetes, cancer and AIDS (Hela and Abdullah, 2010). In normal metabolic condition, oxidant and antioxidant levels are maintained in balance within humans for sustaining optimal physiological conditions (Temple, 2000). 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 diseases (Suja et al., 2004). According to the estimates of the WHO, more than 80.0% of people in developing countries depend on traditional medicine for their primary health needs. A recent survey shows that more than 60% of cancer patients use vitamins or herbs as therapy (Madhuri and Pandey, 2008; Sivalokanathan et al., 2005).

The extensive use of herbal preparations and medicinal plants has provided some of the most important sources of lead compounds for the pharmaceutical industries. Furthermore, over a 100 new products are in the process of clinical development, particularly as anti-cancer agents and anit-infectives (Hafidh et al., 2009). It has been shown that antioxidant rich diets can reduce oxidative damage to DNA, thus preventing a critical step in the onset of carcinogenesis and the impact of antioxidants on mutagenesis and carcinogenesis has been well established (Zhang et al., 2008; Meyskens et al., 2005). Various free radicals are also responsible for the induction of short term algesia (Chung, 2004). Moreover, free radicals play an important role in the pathogenesis of inflammation (Winrow et al., 1993). For several years, many researchers have been investigated powerful and nontoxic antioxidants from natural sources, especially edible or medicinal plants to prevent the reactive species related disorders in human as well as replace the synthetic compounds which are in use may have carcinogenic activity and harmful to the lungs and liver (Rechner et al., 2002).

Glochidion was regarded as a genus of the family Euphorbiaceae, which consists of monoecious, rarely dioecious trees or shrubs. But molecular phylogenetic studies have shown that *Phyllanthus* is paraphyletic over *Glochidion*. A recent revision of the family Phyllanthaceae has subsumed *Glochidion* into *Phyllanthus* (Hoffmann et al., 2006). *Glochidion lanceolarium*(Roxb) Voigt, locally known as Kechchua, Bhauri; Kakra, Anguti (Family-Euphorbiaceae) is evergreen shurb or tree, usually 1-3m tall, rarely 7-12m tall, monoecious, glabrous throughout except hairy ovary and capsule. It grows in Cox's Bazar hillside of Bangladesh. It is also grown in India, China and Hong Kong (Hossain et al., 2004). Traditionally many *Phyllanthus* species are used in haemorrhoids, diarrhoea, dysentery, anaemia, jaundice, dyspepsia, insomnia etc. and some of them can induce diuresis (Ghani, 1998). Many secondary metabolites were isolated from *Glochidion* species, including tannins (Chen et al., 1995), glycosides (Otsuka et al., 2003), lignans (Otsuka et al., 2000), terpenoids (Hui and Li, 1976). Literature reviews indicated that no studies combining the activity of the antioxidant, antitumor, analgesic and anti-inflammatory activities of *G. lanceolarium* have so far been undertaken. Taking this in view and as a part of our ongoing research on Bangladeshi medicinal plants, the present study aimed to evaluate the antioxidant, antitumor, analgesic and anti-inflammatory activities of the methanol and its chloroform and petroleum ether fraction of stem bark of *G. lanceolarium.*

Methodology

Plants materials: The stem bark of *G. lanceolarium* was collected from Mirpur, Dhaka in the month of April, 2009 and identified by Mr. Sarder Nasir Uddin, Scientific Officer, Bangladesh National Herbarium, Dhaka, where a voucherspecimen (DACB-34199) representing this collection has been deposited.

Chemicals: Sodium chloride, propylene glycol, trypan blue, methylene blue, methyl violet and sodium sulphate, were purchased from Merck Limited, Mumbai, India. Diclofenac-Na and Indomethacin were collected from Square Pharmaceuticals Ltd., Bangladesh. All other chemicals and reagents were used of highest analytical grade.

Preparation of extract: The air dried powdered plant material (900 g) was successively cold extracted with methanol (7 days) at room temperature with occasional shaking and stirring. The extractives were filtered through fresh cotton plug and followed by Whatman no. 1 filter paper. The filtrate were then

concentrated by a Buchii rotavapor at low temperature and pressure and afforded methanol (MEGL) extract (36.8199g). The cold methanol extract (10 g) was subjected to Solvent-Solvent partitioning using the protocol designed by Kupchan and modified by Wagene (Wagenen et al., 1933). The extract was partioned successively with petroleum ether (PEFGL) and carbontetrachloride (CTFGL).

Animal: 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 six animals which were fasted overnight prior to the experiments. 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 (Lorke, 1983).

***In vitro* antioxidant activity**

Free Radical Scavenging Activity by 1,1-diphenyl-2-picryl-hydrazyl (DPPH) assay: The free radical scavenging activity of methanol extract and its all fractions, based on the scavenging of the stable 1,1-diphenyl-2- picryl-hydrazyl (DPPH) free radical was determined by the method described by Braca (Braca et al., 2001). The percentage inhibition was calculated from $[(A_0–A_1)/A_0]$ x 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 (µg/ml) versus % inhibition.

***In vivo* antitumor activity**

Transplantation of tumor: Ehrlich ascites carcinoma (EAC) cells were obtained from Indian Institute of Chemical Biology (IICB), Calcutta, 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: 72 Swiss albino mice were divided into six 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 i.p.) and Group-II served as EAC control. After 24-h of EAC transplantation, Group-III, Group-IV and Group-V received MEGL, PEFGL and CTFGL of *G. lanceolarium* stem bark at 20 mg/kg i.p. for nine consecutive days, respectively. Group-VI received reference drug Bleomycin (0.3 mg/kg i.p) for nine consecutive days (Rana and Khanam, 2002). 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/ fractions of *G. lanceolarium* were measured in EAC 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 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: Cell count = (Number of cells × dilution factor)/ (Area × 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) (Sur and Ganguly, 1994).

Hematological parameters: Collected blood was used for the estimation of hemoglobin (Hb) content, red blood cell (RBC) and white blood cell count (Armour et al., 1965).

Analgesic activity

Tail Flick Test: The animals were divided into five groups with six mice in each group. Group I animals received vehicle (1% Tween 80 in water, 10 mL kg^{-1} body weight), animals of Group V received Diclofenac-Na at 10 mg kg^{-1} body weight while animals of Group II, Group III and Group IV were treated with 100 mg kg^{-1} body weight (p.o.) of the MEGL and PEFGL and CTFGL of *G. lanceolarium*. From 1-2 cm of the tail of mice was immersed in warm water kept at constant temperature of 60°C. The reaction time was the time taken by the mice to deflect their tails. The first reading was discarded and the reaction time was recorded as a mean of the next three readings. A latency period of 20 second was defined as complete analgesia and the measurement was then stopped to avoid injury to mice. The latent period of the tail-flick response was determined before and 0, 30, 60 and 90 min after the oral administration of drugs (Toma et al., 2003).

Acetic Acid-Induced Writhing Test: The analgesic activity of the samples was also studied using acetic acid-induced writhing model in mice. Test samples and vehicle were administered orally 30 min before intraperitoneal administration of 0.7% acetic acid but Diclofenac-Na was administered intraperitonially 15 min before injection of acetic acid. After an interval of 5 min, the mice were observed for specific contraction of body referred to as 'writhing' for the next 10 min (Ghule et al., 2011).

Anti-inflammatory Activity

Carrageenan induced paw edema test in mice: Mice were divided into five groups of six animals each. The test groups received 100 mg/kg, *p.o.* of each extract/fractions. The reference group received indomethacin (10 mg/kg, *p.o.*) while the control group received 3 mL/kg of 1% tween 80 in water. After 1 h, 0.1 mL, 1% w/v carrageenan suspension in normal saline was injected into the sub plantar tissue of the right hind paw (Winter et al., 1962). The paw volume was measured at 1, 2, 3 and 4 h after carrageenan injection using a micrometer screw gauge. The percentage inhibition of the inflammation was calculated from the formula: % inhibition = $(1 - D_t/D_0) \times 100$. Whereas D_0 was the average inflammation (hind paw edema) of the control group of mice at a given time, D_t was the average inflammation of the drug treated (i.e. extract/fractions or reference indomethacin) mice at the same time (Gupta et al., 2005).

Statistical Analysis: Antitumor data are expressed as mean ± 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. Analgesic and anti inflammatory data are expressed as mean ± S.E.M. (n = 6 mice per groups). Statistical significance (p) calculated ANOVA followed by Dunnett's-T test $P<0.01$ and $P<0.001$ were considered to be statistically significant.

Results

Acute toxicity studies: 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/fractions of *G.lanceolarium* was safe up to a dose of 500 mg/kg (p.o.) body weight which agrees with the previous study (Rahman et al., 2011). Behavior of the animals was closely observed for the first 3h then at an interval of every 4h during the next 48 h. 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.

DPPH radical scavenging activity: The percentage (%) scavenging of DPPH radical was found to be concentration dependent i.e concentration of the extract/fractions between 0.976-500 µg/ml greatly increasing the inhibitory activity. The IC_{50} value of MEGL and PEFGL and CTFGL were 18.32 ± 0.76 µg/ml, 20.29 ± 0.79 µg/ml, 22.24 ± 2.02 µg/ml, respectively. While IC_{50} value of standard tert- butyl-1-hydroxy toluene was found to be 15.24 ± 0.63µg/ml (Table 1).

Table 1: IC_{50} values of test samples of *G. lanceolarium* and standard.

Test samples/Standard	MEGL	PEFGL	CTFGL	TBHT
IC_{50} (µg/ml)[#]	18.32 ± 0.76	20.29 ± 0.79	22.24 ± 2.02	15.24 ± 0.63

TBHT - tert-butyl-1-hydroxytoluene.

Tumor growth and survival parameters: Antitumor activity of extract/fractions 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 control animals when compared with normal saline control animals. Administration of MEGL and PEFGL and CTFGL at a dose of 20mg/kg significantly ($p<0.05$) decreased the tumor volume, tumor weight and viable cell count. Furthermore, the median survival time was increase to 25 ± 0.32 (% ILS = 24.6), 26± 0.10 (% ILS = 30.3) and 23 ± 0.19 (% ILS = 20.5) on administration of MEGL and PEFGL and CTFGL at a dose of 20 mg/kg, respectively.

Table 2: Effect of MEGL and PEFGL and CTFGL of *G. lanceolarium* 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**	**MEGL**	**PEFGL**	**CTFGL**	**Bleomycin**
Tumor volume (ml)	3.1 ± 0.21	0.79 ± 0.19	0.70 ± 0.14*	0.85 ± 0.41*	0.51 ± 0.21*
Tumor weight (g)	3.90 ± 0.24	1.32 ± 0.29	1.05 ± 0.11*	1.81 ± 0.31*	0.61 ± 0.11*
MST (days)	20 ± 0.12	25 ± 0.32	26 ± 0.10	23 ± 0.19	42.6 ± 0.12
% ILS	00.0	24.6	30.3	20.5	98.81
Viable cell (x 10^7 cell/ml)	8.1 ± 0.22	0.91 ± 0.32*	0.64 ± 0.15*	1.02 ± 0.25*	0.5 ± 0.05*
Non-viable cell (x 10^7 cell/ml)	0.5 ± 0.24	2.17 ± 0.23*	2.25 ± 0.59*	2.17 ± 0.15*	3.3 ± 0.05*
Total cell (x 10^7 cell/ml)	8.6 ± 0.15	3.08 ± 0.35	2.89 ± 0.21	3.19 ± 0.25	3.8 ± 0.05*
Viable %	94.18	29.55	22.15	31.98	13.15
Non-viable %	5.82	70.45	77.85	68.02	86.85

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

Hematological parameters: Hematological parameters (Table 3) of tumor bearing mice on 14 days were found to be significantly altered compared to the normal saline 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 interval on extract/fractions at a dose of 20 mg/kg restored all the altered hematological parameters to almost near normal.

Table 3. Effect of MEGL and PEFGL and CTFGL of *G. lanceolarium* on hematological parameter in EAC bearing mice

Treatment	**RBC (cell x 10^3/mm^3)**	**WBC (cell x 10^3/mm^3)**	**Hemoglobin (g %)**
Normal saline control	5.69 ± 0.12	3.92 ± 0.32	13.90 ± 3.1
EAC control	3.91 ± 0.80*	5.94 ± 0.52*	4.95 ± 1.80*
Bleomycin (10 mg/kg)	5.18 ± 0.12**	3.35 ± 0.83**	12.89 ± 2.93**
MEGL	4.51 ± 0.31**	4.38 ± 0.49*	8.01 ± 2.19**
PEFGL	4.91 ± 0.71**	4.01 ± 0.11**	8.99 ± 1.90**
CTFGL	4.12 ± 0.15*	4.91 ± 0.91**	7.93 ± 2.01**

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

Tail flick test: The tail withdrawal reflex time following administration of the MEGL, PEFGL and CTFGL were found to statistically significant (*p<0.01 and **p<0.001) and was comparable to the reference drug Diclofenac-Na (Table 4).

Table 4. Effects of the *G. lanceolarium* on tail withdrawal reflex of mice induced by tail flick method.

Treatment Group	**Dose (mg/kg body wt.)**	Tail flick time			
		0 Min	30 Min	60 Min	90 Min
Vehicle control (1% tween- 80)	-	1.30±0.11	1.35±0.12	1.43±0.05	1.40±0.11
Standard (Diclofenac- Na)	10	1.40±0.02	2.26±0.25**	3.13±0.10**	3.06±20**
MEGL	100	1.35±0.25	1.53±0.10**	2.30±0.15**	1.11±0.03*
PEFGL	100	1.30±0.25	2.15±0.42**	2.43±0.5**	1.57±0.25*
CTFGL	100	1.30±0.1	1.43±0.01**	2.33±0.5**	1.07±0.16*

Values are mean±SEM, (n = 6);*p<0.01 and **p<0.001, Dunnett's-T test as compared to control.

Writhing test method: Table 5 shows the effects of the extract/fractions on acetic acid-induced writhing in mice. The oral administration of extract/fractions significantly (*p<0.01 and **p<0.001) inhibited (% inhibition of writhing of MEGL, PEFGL and CTFGL were 54.54, 39.09 and 68.18, respectively) writhing response induced by acetic acid.

Table 5: Effects of the *G. lanceolarium* on acetic acid-induced writhing in mice.

Treatment Group	Dose (mg/kg)body wt.	Writhing no.	% Inhibition of Writhing no.
Vehicle control (1% tween 80)		110.0±10.23	-
Standard (Diclifenac-Na)	10	15.0±3.25**	86.36
MEGL	100	50.0 ± 7.15**	54.54
PEFGL	100	67.0± 8.48**	39.09
CTFGL	100	35.0 ±6.57**	68.18

Values are mean±SEM, (n = 6);*p<0.01 and **p<0.001, Dunnett's-T test as compared to control.

Anti- inflammatory activity: To the carrageenan induced paw edema in mice, the extract/fraction showed inhibition on paw edema compared to the control group (Table 6).

Table 6. Effects of the G. lanceolarium on carrageenan induced paw edema in mice.

Treatment Group	Dose (mg/Kg body wt.)	Paw edema volume (cm)			
		1H	2H	3H	4H
Vehicle control (1% tween 80)		1.23±0.11	1.4±0.12**	1.53±0.05**	1.46±0.11.0**
Standard (Indomethacin)	10	1.23±0.023	1.16±0.25**	1.13±0.10**	1.06±0.20**
MEGL	100	1.45±0.07	1.25±0.06**	1.05±0.07**	0.95±0.05**
PEFGL	100	1.25±0.5	1.23±0.5**	1.1±0.12.0**	1.0±0.10.0**
CTFGL	100	1.35±0.02	1.1±0.07**	1.0±0.02**	0.85±0.07**

Values are mean±SEM, (n = 6);*p<0.01 and **p<0.001, Dunnett's-T test as compared to control.

Discussion

The model of scavenging of the stable DPPH radicals is a common method to estimate the antioxidant activity of the investigated samples**.** The effect of antioxidants on DPPH radical scavenging is thought to be due to their hydrogen donating ability. DPPH is a stable free radical

and accepts an electron or hydrogen radical to become a stable diamagnetic molecule (Gulcin et al., 2004). The addition of the investigated extract/fractions to the DPPH solution caused a decrease in the optical density at 517 nm (the maximum absorption of a stable DPPH radical at 517 nm). The decrease in absorbance of DPPH radical caused by antioxidants is due to the reaction between antioxidant molecules and radical which results in the scavenging of the radical by hydrogen donation. This is visualized as a discoloration from purple to yellow.

A regular and rapid increase in ascetic tumor volume (EAC tumor bearing mice) was observed. Ascetic fluid is the straight nutritional source for tumor cells. A rapid increase in ascetic fluid with tumor growth would be a means to meet the nutritional requirement of tumor cells (Prasad and Giri, 1994). Treatment with extract/fractions reduced the intraperitonial tumor burden, thereby reducing the tumor weight, cell growth and increased the life span of the tumor bearing mice. It can therefore be inferred that extract/fractions increased the life span of EAC bearing mice may be by decreasing the nutritional fluid volume and delaying the cell division (Sur et al., 1997). Reduction in viable cell count and increased non viable cell count in groups treated with extract/fractions, towards normalization in tumor host suggests its anti-tumor effect against EAC cell in mice. These tumor cells absorb the anticancer drug in peritoneal cavity and causes lysis of the cells by cytotoxic mechanism (Kennedy et al., 2001). Anemia encountered in ascites carcinoma mainly due to iron deficiency, either by haemolytic or myelopathic conditions which finally lead to reduced RBC number (Gupta et al., 2007). Treatment with extract/fractions brought back the hemoglobin content, RBC and WBC count more or less towards normal levels, thus supporting its haematopoietic protecting activity without inducing myelotoxicity, the most common side effects of cancer chemotherapy.

Previous study of *Glochidione* species indicated the presence of tannin, glycosides, lignan and terpenoid. A number of scientific reports indicate that certain terpenoids, steroids and phenolic compounds such as tannins, caumarins and flavonoids have a chemo preventive role in cancer through their effects on signal transduction in cell proliferation and angiogenesis (Blois, 2002). Thus, it can be thought that the plant extract/fractions exhibited antitumor activity through the combination of these antitumor phytochemicals.

The tail flick method is generally used for evaluating central antinociceptive response. The method is further distinguished by their tendency to respond to the pain stimuli conducting through neuronal pathways as tail immersion mediates a spinal reflex to nociceptive stimuli (Chapman et al., 1985). On the other hand, acetic acid induced writhing response is a sensitive procedure to evaluate peripherally acting analgesics and represents pain sensation by triggering localized inflammatory response. Such pain stimulus leads to the release of free arachidonic acid from the tissue phospholipid (Ahmed et al., 2006). The response is thought to be mediated by peritoneal mast cells (Ribeiro et al., 2000), acid sensing ion channels (Voilley, 2004) and the prostaglandin pathways (Hossain et al., 2006). The organic acid has also been postulated to act indirectly by inducing the release of endogenous mediators, which stimulates the nociceptive neurons that are sensitive to NSAIDs and narcotics (Adzu et al., 2003).

Taken all together, this is an indication that the extract/fractions of *G. lanceolarium* can be used to manage central as well as peripheral pain. However, Kerstein, Camino, Moran, & Stucky (Kerstein et al., 2009) suggest that, the inhibitory effect to pain response is due to inhibit the increase of the intracellular Ca^{2+} ion through the TRPA1 (Transient Receptor Potential Ankyrin 1), a member of the transient receptor potential family of cation channel that trigger the analgesic action. So it is likely that the extract/fractions may contain substances that affect the metabolism of Ca^{2+} ions. Tannins are also found to have a contribution in antinociceptive activity (Ramprasath et al., 2006). So it can be assume that cyclooxygenase (COX) inhibitory activity along with antioxidant activity

may reduce the production of free arachidonic acid from phospholipid or may inhibit the enzyme system responsible for the synthesis of prostaglandins and ultimately relieve pain sensation.

Carrageenan induced oedema has been commonly used as an experimental animal model for acute inflammation and is believed to be biphasic. The early phase (1 – 2 h) of the carrageenan model is mainly mediated by histamine, serotonin and increased synthesis of prostaglandins in the damaged tissue surroundings. The late phase is sustained by prostaglandin release and mediated by bradykinin, leukotrienes, polymorphonuclear cells and prostaglandins produced by tissue macrophages (Kaushik et al., 2012). Since the extract/fractions significantly inhibited paw edema induced by carrageenan in the second phase and this finding suggests a possible inhibition of cyclooxygenase synthesis by the extract/fractions and this effect is similar to that produced by non-steroidal anti-inflammatory drugs such as indomethacin, whose mechanism of action is inhibition of the cyclooxygenase enzyme.

Conclusion

Based on the results of the present study, we conclude that the plant extract/fractions possess antioxidant, antitumor, analgesic and anti inflammatory potential. However, further studies are necessary to examine underlying mechanisms of these effects and to isolate the active compound(s) responsible for these pharmacological activities.

Conflict of interest statement: *We declare that we have no conflict of interest.*

Acknowledgements: *The authors are thankful to the Department of Pharmaceutical Chemistry, University of Dhaka, Bangladesh, Department of Applied Chemistry & Chemical Engineering, University of Rajshahi, Rajshahi, Bangladesh and Department of Pharmacy, BRAC University, Dhaka, Bangladesh for providing laboratory facilities to carry out this research work. The Bose Centre for Advanced Study and Research in Natural Sciences, University of Dhaka, Bangladesh is gratefully acknowledged for partial financial support to carry out the research work.*

References

Adzu B, Amos S, Kapu SD, Gamaniel KS (2003). Anti-inflammatory and anti-nociceptive effects of *Sphaeranthus senegalensis*. J. Ethnopharmacol. 84: 169-174.

Ahmed F, Hossain MH, Rahman AA, Shahid IZ (2006). Antinociceptive and sedative effects of the bark of *Cerbera odollam* Gaertn. J. Ori. Pharm. Exp. Med. 6: 344-348.

Armour DFE, Blood FR, Belden DA (1965). The Manual for laboratory works in mammalian physiology. University of Chicago Press, U.S.A.

Blois MS (2002). Antioxidant determination by the use of a stable free radical. Nature 26:199-200.

Braca A, Nunziatina DT, Lorenzo DB, Cosimo P, Mateo P, Ivano M (2001). Antioxidant principles from *Bauhinia terapotensis*. J. Nat. Prod. 64: 892-895.

Chapman CR, Casey KI, Dubner R, Foley KM, Gracely RH, Reading AE (1985). Pain measurement: an overview. Pain 22: 1-31.

Chen LG, Yang LL, Yen KY, Hatano T, Yoshida T, Okuda T (1995). Tannins of euphorbiaceous plants. XIII: New hydrolyzable tannins having phloroglucinol residue from *Glochidion rubrum* BLUME. Chem. Pharm. Bull. 43: 2088-2090.

Chung JM (2004). The role of reactive oxygen species (ROS) in persistent pain. Mol. Intev. 4: 248-250.

Ghani A (1998). Medicinal plants of Bangladesh: Chemical constituents and uses. Asiatic Society of Bangladesh, Dhaka.

Ghule RS, Venkatnarayanan R, Thakare SP, Jain H, Ghule PR (2011). Analgesic activity of *Cuscuta campestris* Yuncker a parasitic plant grown on *Nerium indicum* Mill. J. Adv. Phar. Educ. & Res. 1: 45-51.

Gulcin IG, Sat SB, Elmastas M, Kufreviouglu OI (2004). Comparison of antioxidant activity of Clove (*Eugenia caryophylata* Thunb) buds and lavender (*Lavandula stoechas* L). Food Chem. 87: 393-400.

Gupta M, Mazumder UK, Halder PK, Kandar CC, Monikandan L, Senthil GP (2007). Anticancer activity of *Indigofera aspalathoides* and *Wedelia calendulaceae* in Swiss albino mice. Iran. J. Pharm. Res. 6: 141-45.

Gupta M, Mazunder UK, Sambath KR, Gomath P, Rajeshwar Y, Kakoti BB (2005). Anti-inflammatory, analgesic and antipyretic effects of methanol extract from *Bauhinia racemosa* stem bark in animal models. J. Ethnopharmacol. 98: 267-273.

Hafidh RR, Abas F, Abdulamir AS, Jahanshiri F, Bakar FA, Sekawi Z (2009). A review: Cancer research on natural products in Asia. Int. J. Cancer Res. 5: 69-82.

Hela AE, Abdullah A (2010). Antioxidant and antimicrobial activities of methanol extracts of some Verbena species: *In vitro* evaluation of antioxidant and antimicrobial activity in relation to polyphenolic content. J. Appl. Sci. Res., 6: 683-9.

Hoffmann P, Kathriarachchi H, Wurdack KJ (2006). A phylogenetic classification of Phyllanthaceae (Malpighiales; Euphorbiaceae *sensu lato*). Kew. Bull. 61: 37-53.

Hossain KM, Rahman ML, Hoque ATMR, Alam MK (2004). Comparative regeneration status in a natural forest and enrichment plantations of Chittagong (south) forest division, Bangladesh. J. Forestry Res. 15: 255-260.

Hossain MM, Ali MS, Saha A, Alimuzzaman M (2006). Antinociceptive activity of whole plant extracts of *Paederia foetida*. Dhaka Univ. J. Pharm. Sci. 5: 67-69.

Hui W-H, Li M-M (1976). Lupene triterpenoids from *Glochidion eriocarpum.* Phytochemistry 15: 561-562.

Kaushik D, Kumar A, Kaushik P, Rana AC (2012). Analgesic and antiinflammatory activity of *Pinus roxburghii* Sarg. Adv. Pharmacol. Sci. doi:10.1155/2012/245431.

Kennedy DO, Kokima A, Hasuma T, Yano Y, Otani S, Matsui-Yuasa I (2001). 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: 113-33.

Kerstein PC, del Camino D, Moran MM, Stucky CL (2009). Pharmacological blockade of TRPA1 inhibits mechanical firing in nociceptors. Mol. Pain 5: 19-25.

Lorke DA (1983). A new approach to practical acute toxicity testing. Arch. Toxicol. 54: 275-287.

Madhuri S, Pandey G (2008). Some dietary agricultural plants with anticancer properties. Plant. Arch. 8: 13–16.

Meyskens FL, Szabo E (2005). Diet and cancer: the disconnect between epidemiology and randomized clinical trials. Cancer Epidemiol. Biomarkers 14: 1366-1369.

Otsuka H, Hirata E, Shinzato T, Takeda Y (2000). Isolation of lignan glucosides and neolignan from the leaves of *Glochidion zeylanicum* (Gaertn) A. Juss. Chem. Pharm. Bull. 48: 1084-1086.

Otsuka H, Kijima H, Hirata E, Shinzato T, Takushi A, Bando M, Takeda Y (2003). Glochidionionosides A-D: megastigmane glucosides from leaves of *Glochidion zeylanicum* (Gaertn.) A. Juss. Chem. Pharm. Bull. 51: 286-290.

Prasad SB, Giri A (1994). Antitumor effect of cisplatin against murine ascites Dalton's lymphoma. Indian J. Exp. Biol. 32: 155-62.

Rahman MH, Alam MB, Chowdhury NS, Jha MK, Hasan M, Khan MM, Rahman MS, Haque ME (2011). Antioxidant, analgesic and toxic potentiality of *Stephania japonica* (Thunb.) Miers. Leaf. Int. J. Pharmacol. 7: 257-62.

Ramprasath VR, Shanthi P, Sachdanandam P (2006). Immunomodulatory and anti- inflammatory effects of *Semecarpus anacardium* LINN. nut milk extract in experimental inflammatory conditions. Biol. Pharm. Bull. 29: 693-700.

Rana AYKMM, Khanam JA (2002). *Aristolochia indica* whole plant extract as an antineoplastic agent. J. Med. Sci. 2: 202-205.

Rechner AR, Kuhnle G, Bremner P, Hubbard GP, Moore KP, Rice-Evans CA (2002). The metabolic fate of dietary polyphenols in humans. Free Radic. Biol. Med. 33: 220-235.

Ribeiro RA, Vale ML, Thomazzi SM, Paschoalato ABP, Poole S, Ferreira SH, Cunha FQ (2000). Involvement of resident macrophages and mast cells in the writhing nociceptive response induced by zymosan and acetic acid in mice. Eur. J. Pharmacol. 387: 111-118.

Sivalokanathan S, Ilayaraja M, Balasubramanium MP (2005). Efficacy of *Terminalia arjuna* (Roxb.) on N-nitrosodiethylamine induced hepatocellular carcinoma in rats. Indian J. Exp. Biol. 43: 264–67.

Suja KP, Jayalekshmy A, Arumughan C (2004). Free radical scavenging behavior of antioxidant compounds of sesame (*Sesamum indicum* L.) in DPPH center dot system. J. Agric. Food. Chem. 52: 912-915.

Sur P, Ganguly DK (1994). Tea plant roots extract (TRE) as an antineoplastic agent. Planta. Med. 60: 106-109.

Sur P, Bag SP, Khanam JA (1997). Choroacetohydroxamic acid as antitumor agent against Ehrlich ascites carcinoma in mice. Neoplasma 44: 197-201.

Temple NJ (2000). Antioxidants and disease: more questions than answers. Nutr. Res. 20: 449-459.

Toma W, Graciosa JS, Hiruma-Lima CA, Andrade FDP, Vilegas W, Souza-Brita ARM (2003). Evaluations of the analgesic and antiedematogenic activities of *Quassia amara* bark extract. J. Ethnopharmacol. 5: 19-23.

Voilley N (2004). Acid-sensing ion channels (ASICs): New targets for the analgesic effects of Non-Steroid Anti-Inflammatory Drugs (NSAIDs). Curr. Drug Targets Inflamm. Allergy. 3: 71-79.

Wagenen BC, Larsen RJS, Cardellina HD, Randazzo ZCI, Justisatienr A (1933). Ulosantion, a potent insecticide from the sponge *Ulosa ruetleri*. Organic Chem. 58: 335-337.

Winrow VR, Winyard PG, Morris CJ, Blake DR (1993). Free radicals in inflammation: second messengers and mediators of tissue destruction. Br. Med. Bull. 49: 506-522.

Winter CA, Risley EA, Nuss GW (1962). Carrageenan induced oedema in hind paw of the rats as an assay for anti-inflammatory drugs. Pro. Soc. Exp. Biol. Med. 111: 544-547.

Zhang Y, Seeram NP, Lee R, Feng L, Heber D (2008). Isolation and identification of strawberry phenolics with antioxidant and human cancer cell antiproliferative properties. J. Agric. Food Chem. 56: 670-675.