Journal of
Fundamental and Applied Pharmaceutical Science
https://journal.umy.ac.id/index.php/jfap

Studies on Antioxidant and Antineoplastic Potentials of *Oldenlandia corymbosa* Linn. Leaves

Md. Julkar Nime[1,4], Md. Habibur Rahman[1], Ziasmin Khatun[1,2], Md. Rowshanul Habib[1], Ayesha Siddika[1,3], Marina Khatun[1,5], Md. Rezaul Karim[1], Md. Abdul Aziz[1]*

[1]Department of Biochemistry and Molecular Biology, University of Rajshahi, Rajshahi, Bangladesh
[2]Department of Biochemistry and Biotechnology, University of Barishal, Barishal, Bangladesh
[3]Institute of Tissue Banking and Biomaterial Research, Atomic Energy Research Establishment (AERE), Dhaka, Bangladesh
[4]Biotechnology Research Center and Department of Biotechnology, Toyama Prefectural University, Toyama, Japan
[5]Department of Biochemistry and Molecular Biology, Mawlana Bhashani Science and Technology University, Tangail, Bangladesh

Abstract

Various bioactive properties, including antioxidant and antineoplastic properties, are extracted from plant species. *Oldenlandia corymbosa* Linn. is an important medicinal plant that grows as a weedy annual herb throughout Bangladesh. However, no previous studies have reported the antineoplastic and antioxidant potentials of *Oldenlandia corymbosa* leaves. Based on this fact, this investigation evaluated the methanol extract of *Oldenlandia corymbosa* Linn. leaves (MELO) for its antioxidant and antineoplastic properties. Different *in vitro* experiments were applied here to measure the phytochemical content and antioxidant activity of MELO. Functional assays were utilized to measure the antineoplastic activity of MELO against Ehrlich Ascites Carcinoma (EAC) in mice. MELO contains rich phenolic compounds, flavonoids, flavonol, and proanthocyanidin. *In vitro* antioxidant assay, MELO exhibited significant ABTS▪ (IC_{50}: 98.07 µg/mL) and DPPH (IC_{50}: 151.21µg/mL) radicals scavenging activity when compared with the positive control (Ascorbic acid). MELO also showed total antioxidant and ferrous-reducing properties. In the antineoplastic study, dose-dependent reduction in viable EAC cell count was observed in MELO-treated groups. In contrast, treatment with MELO significantly ($P<0.05$) increased the mean survival time and life span (65.21%) of EAC-bearing mice. The altered hematological parameters of mice of the EAC control group were restored to the normal level by treating MELO. MELO also induced the apoptotic features observed in the morphology of DAPI-stained EAC cells. As identified by GC/MS analysis, the Phytochemical profiles of MELO are also consistent with their antioxidant, antineoplastic and apoptotic properties. Thus, the methanol extract of *Oldenlandia corymbosa* leaves (MELO) has the potential to consider a good source of antioxidants and antineoplastic agents.

Keywords: antineoplastic; antioxidant; leaves; *Oldenlandia corymbosa*; phytochemical

Data of article
Received : 27 Jan 2023
Reviewed : 18 Mar 2023
Accepted : 22 Mar 2023

DOI
10.18196/jfaps.v2i1.17737

Type of article:
Research

* *Corresponding author, e-mail: maazizbio@ru.ac.bd*

INTRODUCTION

Above 80% of the population in developing countries still uses traditional medicine to treat their primary health problems. Currently, plant-based drugs are formulated, modified, and efficiently used in modern drugs. Mostly herbal and some traditionally used medicinal plants are good sources of antioxidant compounds that have the proficiency to neutralize free radicals and reactive oxygen species (ROS). Free radicals have been implicated in the etiology of several major human ailments, including cancer, diabetes, cardiovascular diseases, etc.[1] Phenolics and flavonoids are principal plant-derived bioactive compounds known as natural antioxidants containing their redox properties. Moreover, these compounds act as hydrogen ion donors, free radical scavengers, reducing agents, and chelating of metal ions.[2] Natural antioxidants have obtained great deliberation owing to their efficient prevention of human body-inducing oxidative stress by removal of reactive oxygen species and abate the progress of many chronic diseases such as cancer, type 2 diabetes mellitus, etc.[3] Cancer is a non-communicable disease and continues to represent the second largest cause of the world mortality rate. Developed and developing countries deserve immediate consideration for controlling and preventing cancer because it will be apprehended twice in three decades.[4] Hence, the scientific community utilizes natural products to develop new anticancer agents with fewer side effects.

A fruitful antineoplastic drug should kill or disable cancer cells but not cause damage to normal cells. This ideal situation only happens when an antineoplastic agent can induce apoptosis only in cancer cells. Current studies expose medicinal plants as a source of novel compounds that can bring apoptotic death of cancer cells but not normal cells.[5-7] Therefore, the history of the traditional use of plants has provided valuable guidance in developing the anticancer drug. *Oldenlandia corymbosa* Linn, a diamond flower in Bangladesh, is an important medicinal plant.[8] It belongs to the Rubiaceae family and is used in traditional medicine to treat ailments like jaundice and other liver diseases, heat-eruption, violated pitta conditions, hyperdense, giddiness, dyspepsia, leprosy, other skin diseases, cough and nervous depression.[8] Different biological properties of this plant have also been observed, such as antiulcer,[9] anthelmintic,[10] analgesic,[11] hepatoprotective,[11] effective antimalarial activity,[11] and antibacterial and antifungal activity.[12] The ethanol solvent extract of this plant showed a significant effect on uterine contraction[13] and had significant diuretic activity[14].

However, no published reports disclose the antioxidant and antineoplastic effects of *Oldenlandia corymbosa*. Thus, this study aims to explore the phytoconstituents and antioxidant efficacy of methanolic extract of leaves of *Oldenlandia corymbosa* (MELO) as well as its antineoplastic activity against Ehrlich ascites carcinoma (EAC) in mice.

METHOD

Chemicals and Reagents

DPPH (2,2-diphenyl-1-picrylhydrazyl), ABTS• (2,2′-azino-bis(3-ethylbenzothiazoline-6 sulfonic acid), potassium persulfate, Folin-Ciocalteus's phenol, $AlCl_3$, sodium carbonate were collected from Sigma-Aldrich, Germany. Wako pure chemicals Ltd, Japan, provided catechin and gallic acid. Ascorbic acid (E-Merck, India), Dimethyl sulfoxide (DMSO)

(Labscan, Thailand) and other solvents were analytical grades.

Preparation of methanolic extract from *Oldenlandia corymbosa* Leaves

The plants *Oldenlandia corymbosa* Linn. were collected from the resource area of the Rajshahi University campus in October 2021. The plant was authenticated by the authority of Herbarium, Department of Botany, Rajshahi University, Rajshahi, where voucher specimen No. 27 was deposited for *Oldenlandia corymbosa* Linn. The collected leaves were washed with water and then shade-dried for 7-10 days. After drying, 150 gm of powdered material was immersed in 450 mL methanol at room temperature for 7 days. Then the extract was filtered through a filter paper (Whatman No.1) and concentrated with a rotary evaporator under reduced pressure at 40°C to have 3.5 gm methanol extract of leaves of *Oldenlandia corymbosa* Linn. (designated as MELO).

Quantitative Phytochemical Analysis

Previously described methods were employed to measure the total phenolic (TPC) and flavonoid (TFC) contents of MELO[15,16]. The total phenolics content of MELO was expressed in terms of gallic acid equivalent, GAE (standard curve equation y = 0.0069x + 0.0044; R^2 = 1; mg of GA/g of dry extract). Total flavonoid content was expressed in terms of ascorbic acid equivalent (standard curve equation y = 0.0046x + 0.0871; R^2=0.9894), mg of Ascorbic acid/g of dry extract).

Meanwhile, the total proanthocyanidins and flavonol content of MELO were determined by the previously described method.[17,18] Total proanthocyanidins content was expressed in terms of ascorbic acid equivalent (standard curve equation: y =0.001x -0.0009; R^2=0.9995 mg of ascorbic acid/g of dry extract). Total flavonols content was expressed in terms of Quercetin equivalent, QUE (standard curve equation y = 0.0159x - 0.01; R^2=0.999), mg of QU/g of dry extract.

Determination of Antioxidant Activity

For evaluating the antioxidant activity of MELO, different techniques like DPPH, ABTS, ferric reducing antioxidant capacity (FRAC) and total antioxidant capacity (TAC) assays were applied.

TAC and FRAC of samples or standards were determined as described by Prieto et al.[19] and Oyaizu et al.[20] respectively, with some modifications. Ascorbic acid was used as a reference standard. In TAC and FRAC assays, the absorbance of the reaction mixture was taken at 695 nm and 700 nm, respectively. For both assays, the increment in the absorbance indicated the remarkable antioxidant and reduced MELO capacity.

Free radical scavenging activity was determined by DPPH radical scavenging assay as described by Choi et al.[21] with some modification. In contrast, the antioxidant capacity was estimated regarding the ABTS●+ radical scavenging activity following the procedure described by Cai et al.[22] Ascorbic acid was used as a reference compound. In DPPH and ABTS assays, the absorbance of the final reaction mixture was taken at 517 nm for DPPH and at 734 nm for ABTS assay. The following formulas were used to determine the DPPH and ABTS radical scavenging activities of MELO:

Percentage of free radical scavenging = $[(A_c - A_s)/A_c)] \times 100$ ---------- (1)

Where A_c is the absorbance of the control (without the test sample) and A_s is the absorbance of the test sample.

Gas Chromatography-Mass Spectrometry (GC-MS) Analysis

Gas chromatography/mass spectrometry (GC/MS) was applied to identify the compounds present in MELO, where GCMS-QP2010S (Shimadzu Kyoto, Japan) spectrometer was used. This GCMS spectrometer was equipped with a Rxi-5MS fused silica capillary column (5% diphenyl/95% dimethyl polysiloxane) of 0.25 mm diameter, 30 m length, and 0.25 µm film thickness. The injector temperature was maintained at 270 °C, and the neat injected quantity of sample was 2 µL. Helium was utilized as a carrier gas, and its flow rate was 1.0 mL min^{-1}. Spectra were scanned from 20 to 550 *m/z* at 2 scans s^{-1}. The computer library (NIST) installed in the GC-MS instrument was used to identify the chemical constituents of MELO.

Experimental Animal, Cell Lines, and Ethical Clearance

Swiss Albino male mice of 3-4 weeks of age, weighing 20-27 g, were collected from International Center for Diarrheal Diseases Research, Bangladesh (ICDDR'B). A transplantable tumor (Ehrlich's ascites carcinoma) was used in this thesis from our Research Laboratory at the University of Rajshahi. It was maintained in our laboratory in Swiss Albino mice by intra-peritoneal transplantation bi-weekly. For cancer research, permission and approval were obtained to continue the protocol for using mice as an animal model from the Institutional Ethics Committee for Experimentations on Animal, Human and Microbes and Living Natural Sources (No. 225/320-IAMEBBC/IBSc), Institute of Biological Sciences, University of Rajshahi, Bangladesh.

***In vivo* determination of EAC cell growth inhibition and observation of the Morphological Changes in EAC Cells** [23]

To determine the cell growth inhibition properties of MELO, three groups of Swiss albino mice (n = 8) weighing (25±4) g were used. For the therapeutic evaluation, 6 × 10^5 EAC cells were inoculated into each group of mice on day 0. Treatments were started after 24 hrs of tumor inoculation and continued for 6 days. In brief, the mice in group 1 were considered untreated EAC control, and groups 2 and 3 received MELO at 50 and 100 mg/kg/day, respectively, via intraperitoneal injection. Mice of each group were sacrificed on day 7 after inoculating EAC cells, and the total intraperitoneal cancer cells were harvested with 0.98% normal saline. Viable cells were identified with trypan blue and then counted by a hemocytometer under an inverted microscope (XDS-1R, Optika, and Bergamo, Italy). Cell growth inhibition was calculated by the following formula:

% Cell growth inhibition = {(Cw-Tw) ÷ Cw}×100 ---------- (2)

Where Tw = mean number of tumor cells of treated groups and

Cw = a mean number of tumor cells of control.

During studies on cell growth inhibition, EAC cells were also collected from mice of untreated and MELO-treated groups after sacrificing for observation of morphological changes of a cell by 4,6-diamidino-2-phenylindole (DAPI) staining.

Determination of survival time and average tumor weight

The previous report[24] on EAC cell-bearing mice's survival time and tumor weight was followed for MELO. Three (3) groups were taken, each containing 8 mice. On day zero, 6×10^5 cells/mouse were inoculated into mice of three groups. In this case, mice from group 1 were taken as an

untreated control group. Treatment with MELO was started 24 hours after injection, and mice of groups 2 and 3 received MELO at 50 and 100 mg/kg/mouse/day, respectively. The treatment was continued for 10 days. On the 15th day after EAC cell inoculation, each group's average body weight gain was measured to determine the tumor weight. The survival time was recorded and expressed as mean survival time (MST) in days, and the percent increase of life span (%ILS) was calculated as follows:

MST = (∑ Survival time in days of each mouse in a group) / Total number of mice). ------ (3)

% ILS = (MST of treated group / MST of the control group – 1) x 100 ---------- (4)

Estimation of Hematological Parameters

As described in the previous report[25], four groups (n = 8) of mice were taken to measure the effect of MELO on hematological parameters. 0.2 mL (1.6×10^6 cells/mL) of EAC cells were administered to the mice of all groups except group-1 (designated as normal) at day zero. After 24 hours of inoculation, normal saline (5 mL/kg/mouse/day) was administered (i.p) to normal (group 1) and EAC control (group 2), respectively, for 10 days. Mice in groups 3 and 4 received (i.p) MELO at 50 and 100 mg/kg/mouse/day doses, respectively. On the 12th day after EAC cell inoculation, previously described methods[24] were applied to measure the hematological parameters (Hemoglobin, RBC, and WBC) from freely flowing tail vein blood of mice of different groups.

Statistical Analysis

Data were written as mean ± SD (Standard deviation). One-way analysis of variance (ANOVA) followed by Dunnett's 't' test was applied to conduct statistical analysis. Statistical Package for Social Science (SPSS) software of 21 versions was used for this purpose. $P<0.05$ were considered to be statistically significant.

RESULTS AND DISCUSSION

Phytochemical Constituents of MELO

Nowadays, phenolic compounds such as flavonoids, phenolic acids and tannins possess diverse biological activities like anti-diabetic, anti-inflammatory, anti-carcinogenic and anti-atherosclerotic activities and their effects on human nutrition and health are considerable due to their effectiveness, easy availability, fewer side effects and cost-effectively[26,27]. Polyphenolic compounds show cytotoxicity in various cancer cell lines through different mechanisms[28]. This study has determined the total amount of polyphenolic compounds, flavonoids, flavonols and proanthocyanidins in MELO using various colorimetric methods. The quantitative estimation showed that MELO contained a rich amount of polyphenols content and the appearance of proanthocyanidins and flavonols as the main contents in the obtained extract (Table 1). Thus, the presence of polyphenolic compounds in MELO may be responsible for its antioxidant and antineoplastic activity.

Table 1. Amounts of different Polyphenol constituents in MELO

Phytochemicals	Phenolics	Flavonoids	Proanthocyanidins	Flavonols
MELO	66.07±0.30	43.33 ± 1.64	227.56± 0.57	100.41 ± 0.85

Results were expressed as mean ± SD (n = 3). a, b, c, and d expressed in terms of GAE, AA, AA and QUE, respectively (mg of GAE, AA, AA, and QUE/g of dry extract, respectively).

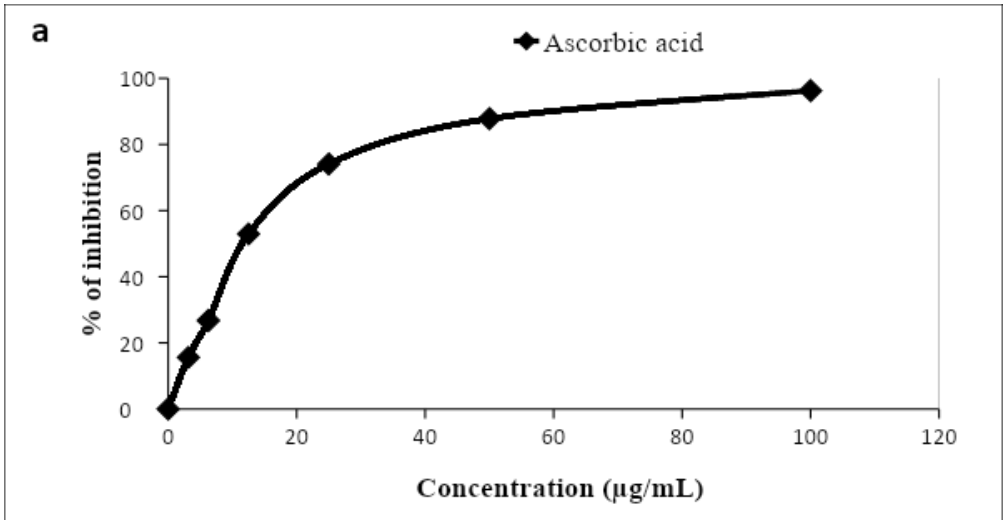
***In vitro* antioxidant activity of MELO**

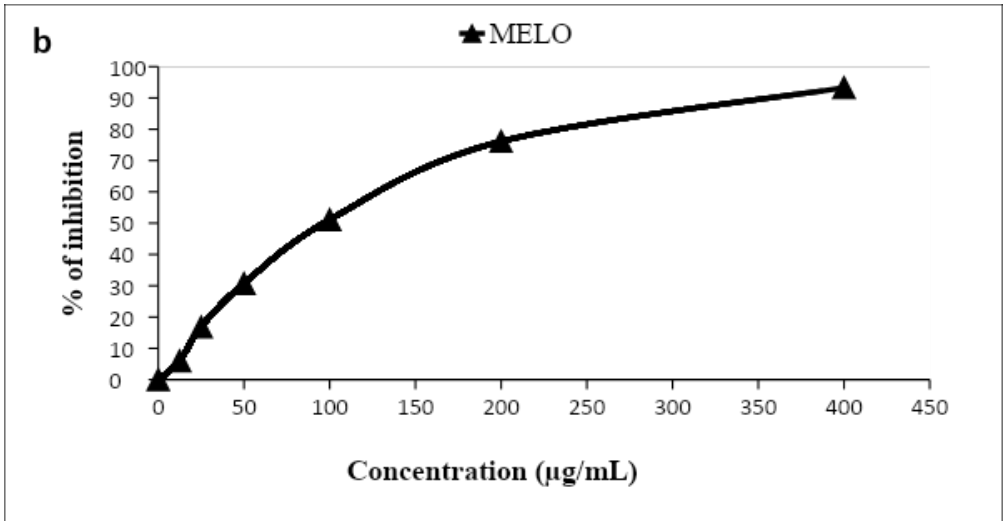
It has been reported that the phenolic compounds are significantly associated with the antioxidant activity of plant extracts mainly for their redox properties, allowing them to act as hydrogen donors, reducing agents, singlet oxygen quenchers, hydroxyl radical quenchers, and metal chelators.[29] As antioxidants, polyphenolic compounds help control life-threatening diseases like cancer by decreasing the overproduction of free radicals and boosting the antioxidant defense mechanism. In this study, the total antioxidant and ferric-reducing antioxidant capacity of MELO was increased along with its concentration. This type of efficacy of MELO was comparable to ascorbic acid (Table 2). MELO exhibited significant concentration-dependent scavenging activity against DPPH (Fig. **1a**) and ABTS (Fig. **1b**) radicals compared to standard. The IC_{50} values of MELO and ascorbic acid (standard) in the DPPH assay were 151.21 and 20.92 µg/mL, whereas, in the ABTS assay, the IC_{50} values were found to be 98.07 and 23.20 µg/mL for MELO and ascorbic acid, respectively. The high phenolic content of MELO can be considered a strong reason for their DPPH and ABTS free radical scavenging activities. The pattern of findings in this study showed similarities with a previous inspection where the antioxidant and total phenolic content of the methanolic leaf extract of *Nyctanthes arborists* L were evaluated.[30]

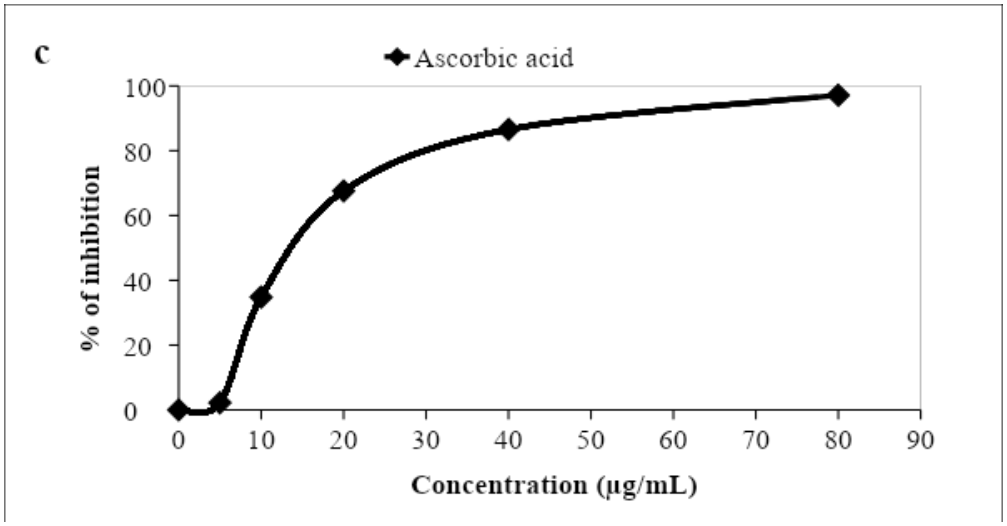
Table 2. Total antioxidant and reducing power capacity of MELO, MESO, and Ascorbic acid

Sample	Concentration(µg/ml)	Total antioxidant activity	Ferrous reducing capacity
Ascorbic acid	3.13	0.047±0.001	0.137±0.001
	6.25	0.111±0.002	0.269±0.002
	12.5	0.245±0.003	0.467±0.003
	25	0.558±0.003	0.860±0.001
	50	1.109±0.002	1.411±0.002
	100	1.812±0.001	2.208±0.004
MELO	25	0.020±0.001	0.012±0.001
	50	0.038±0.001	0.023±0.002
	100	0.067±0.001	0.036±0.001
	200	0.139±0.002	0.089±0.002
	400	0.301±0.002	0.221±0.006
	800	0.567±0.004	0.451±0.002

Data are expressed as mean ± SD.


a
Ascorbic acid
% of inhibition
Concentration (µg/mL)


b
MELO
% of inhibition
Concentration (µg/mL)


c
Ascorbic acid
% of inhibition
Concentration (µg/mL)

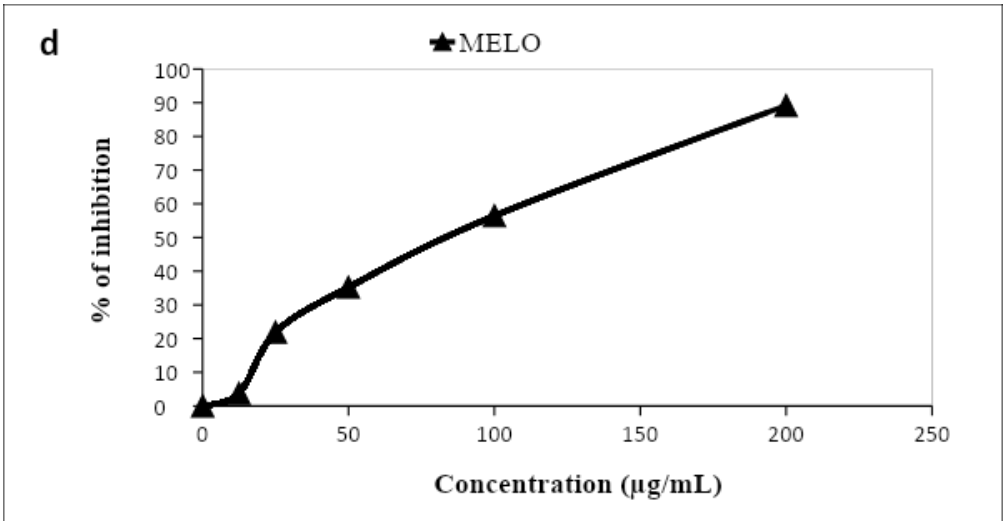


Figure 1. Determinations of DPPH radical scavenging activity of (a) Ascorbic acid, (b) MELO as well as determinations of ABTS+ radical scavenging activity of (c) Ascorbic acid, (d) MELO. Data are expressed as mean ± SD (n = 3) for all experiments. IC_{50} values were calculated from this non-linear regression curve.

Inhibition of EAC cell growth and increment in survival time of EAC cell-bearing mice by MELO

Experimental tumor of EAC cell-bearing mice grows rapidly with noticeable invasive behavior and assimilating human tumors31. The necessary nutritional supplement is provided by ascetic fluid for the proper growth of EAC cells within the peritoneal cavity of mice. Ascitic fluid accumulated in the peritoneal cavity provides nutrition for the growth of EAC cells32. Thus, increasing the volume of ascetic fluid contributes to tumor-bearing mice's body weight gain. For evaluating the antineoplastic effects of an antineoplastic agent, viable EAC cells count and body weight gain were important parameters in vivo tests. In this study, treatment with MELO resulted in significant ($p \leq 0.001$) inhibition of EAC cell growth (Table 3). Treatment with MELO significantly reduced viable EAC cell count when the results were compared with the untreated control. The percentage (%) of cell growth inhibition was found to be 48.64% and 75.44% for MELO at 50 and 100 mg/kg doses, respectively (Table 3). After 15 days of tumor cell inoculation, the average weight gain of the untreated group was 19.80 ± 1.36 g. In contrast, it was 13.7 ± 1.78 g and 9.6 ± 1.10 g ($P<0.05$) for the groups treated with MELO at 50 and 100 mg/kg, respectively (fig. 4). Therefore, treatment with MELO significantly decreased the viable EAC count and body weight gain in respect to untreated control thereby showing its antineoplastic effect. Moreover, for an antineoplastic agent, survival time is an important factor33. An effective antineoplastic agent increases the survival time of cancer-bearing animals. In this work, the MST (Mean survival time) of the untreated control group was 23.13 ± 1.15 days, whereas it was 29.33 ± 1.82 and 38.25 ± 1.89 ($P<0.05$) for the group treated with MELO at 50 and 100 mg/kg (Fig. 2) respectively. It has been observed that the life span of EAC cell-bearing mice was increased when they were treated with MELO at doses 50 and 100 mg/kg doses (Fig. 3) as compared with that of control mice ($p<0.05$). Thus, MELO increased EAC-bearing mice's survival time, indicating its antineoplastic efficacy.

Table 3. Effect of MELO on Ehrlich ascites carcinoma (EAC) cell growth

Group Number	Treatment	Viable EAC cells on day 6 after inoculation (x 10^6 cells/ml)	Percentage (%) cell growth inhibition
1	EAC cell	7.48 ± 1.264	-
2	EAC + MELO (50 mg/kg)	3.84±1.94*	48.641±0.683
3	EAC + MELO (100 mg/kg)	1.83±1.032*	75.445±0.827

Data are expressed as mean ± SD (n = 8); Significance difference concerning EAC control. *P<0.05.

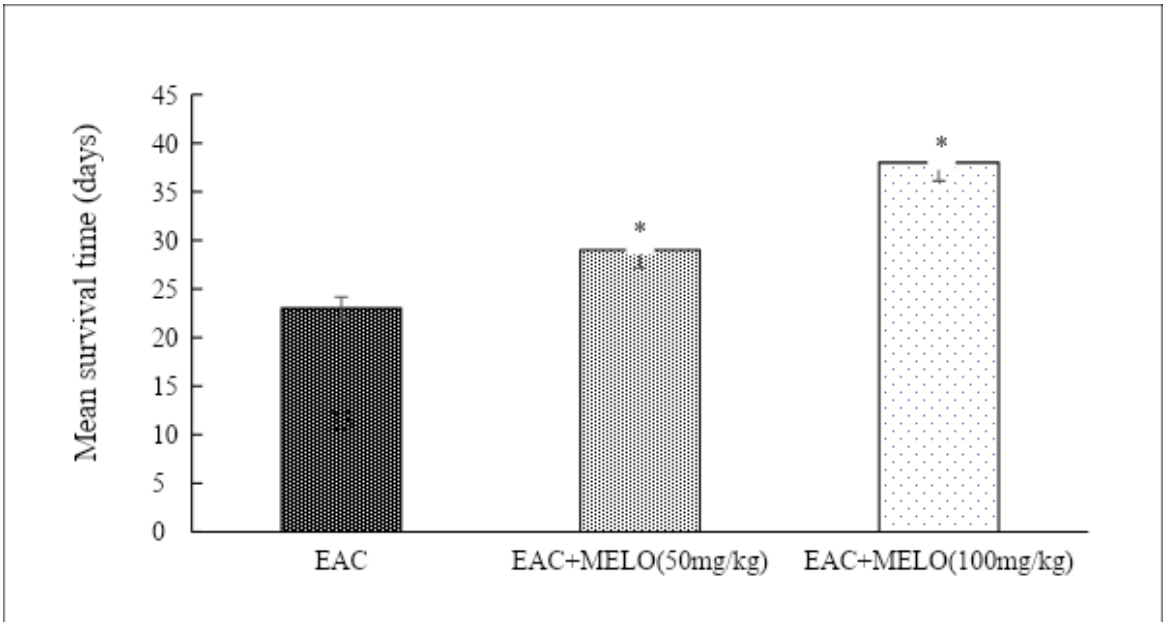


Figure 2. Effect of MELO on EAC cell-bearing mice's mean survival time (MST). Significance difference concerning EAC control. *P<0.05

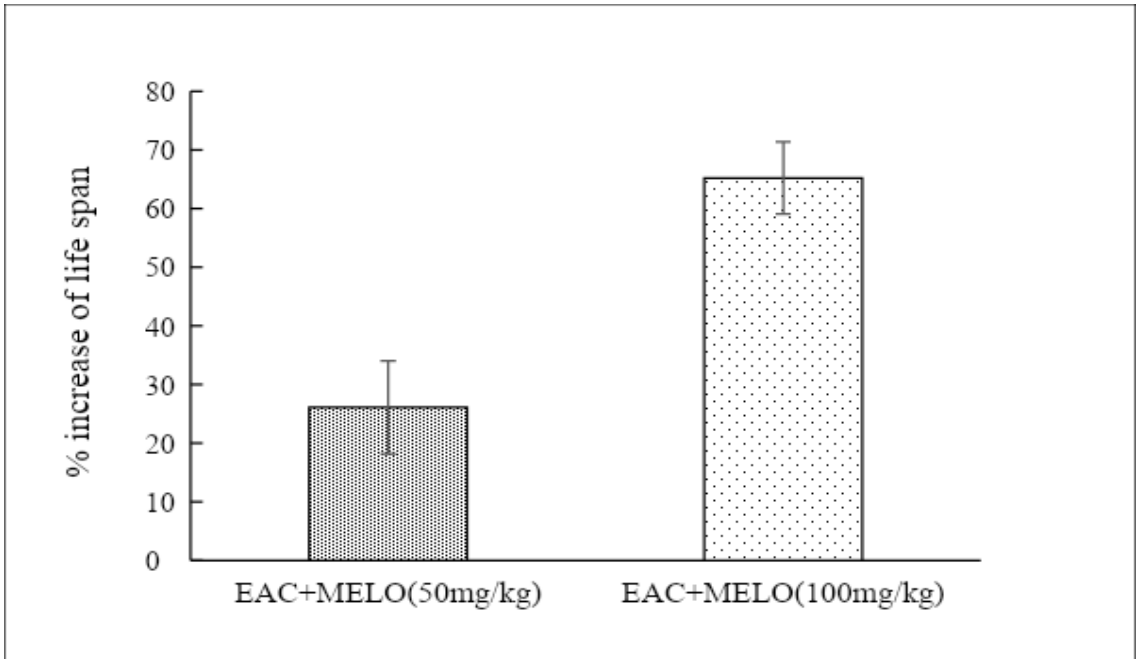


Figure 3. Effect of MELO on percentage increased life span (%ILS)

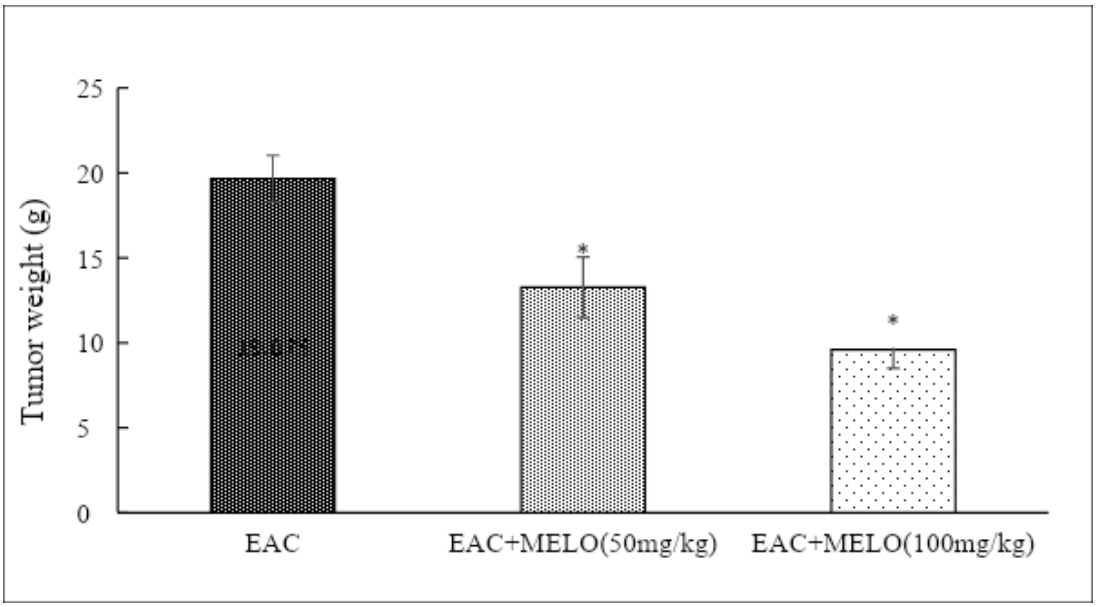


Figure 4. Effect of MELO on tumor weight in tumor-bearing mice after 14 days. Significance difference concerning EAC control. *P<0.05.

Effects of MELO on hematological parameters of EAC cell-bearing mice

Generally, myelosuppression and anemia are found as the major complications during the chemotherapy of a cancer patient[34,35]. In this case, significant alterations were observed in the hematological parameters of mice of the untreated EAC group on day 12 (Table 3). A significant (P<0.05) alteration in hemoglobin level, RBC and WBC count was observed in untreated EAC control group mice compared to normal mice's data. Conversely, treatment with MELO (50 and 100 mg/kg doses) remarkably restored these altered parameters to a more or less normal level.

Table 3. Effect of MELO on blood parameters of tumor-bearing and normal Swiss albino mice

Group Number	Parameters	Hgb (g/dL)	RBC (x10^9 cells/mL)	WBC (x10^6 cells/mL)
1	Normal	14.42 ± 0.51	5.67 ± 0.33	7.73 ± 0.58
2	EAC +Vehicle	9.14 ± 0.90	1.13 ± 0.32	36.08±3.93*
3	EAC + MELO (50 mg/kg)	10.32 ± 2.58	2.03±0.24	23.16 ± 2.56^t
4	EAC + MELO (100 mg/kg)	12.91±1.86	3.96±0.1^t	12.87±3.83^t

Data are expressed as mean ± SD for five animals in each group. *P<0.05: against normal group and ^{t}P≤0.05: against EAC control group.

Induction of apoptotic features in EAC Cells by MELO

Morphological changes of EAC cells were examined by DAPI staining after collecting the cells from non-treated EAC-bearing mice and mice treated with MELO (100

mg/kg/day) for 6 days. It was noted that the normal regular shape of stained EAC cell nuclei was observed in the control group (treated with solvent), as shown in Fig. 5a. In contrast, chromosome condensation, membrane blebbing, apoptotic body, and fragmented DNA were observed in EAC cells treated with MELO (Fig. 5b). These results suggested that MELO treatment could persuade apoptosis in EAC cells.

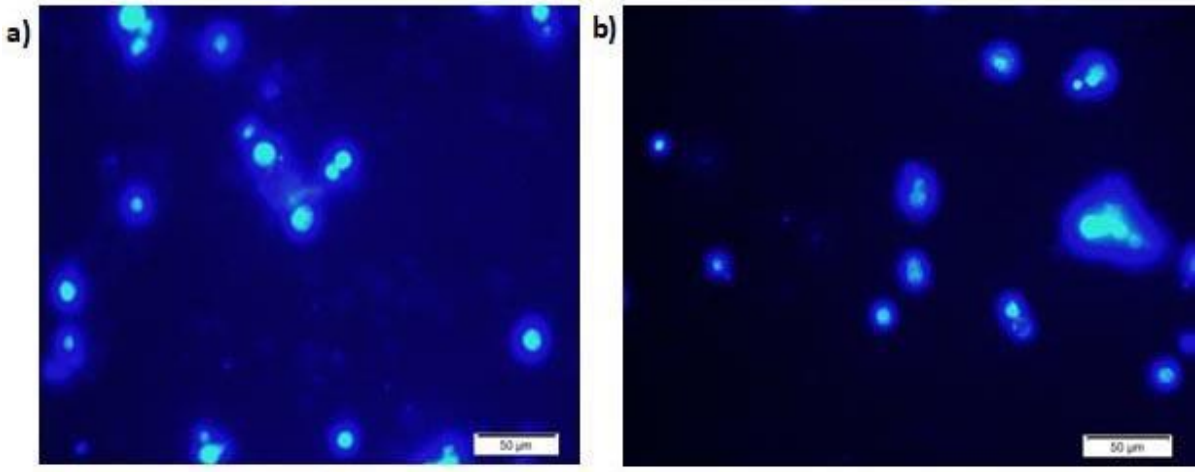


Figure 5. Fluorescence microscopic view of EAC cells collected from the mice of the EAC control group (a) and EAC cells collected from MELO-treated mice at 100 mg/kg (b)

Chemical composition of MELO and MESO

Six compounds were identified in MELO by matching their mass spectrum with the library (NIST) installed in the GC-MS instrument. These compounds accounted for 53.5% of the plant extract (Table 2, Fig 1). The GC-MS chromatogram of MELO identified some compounds (o-Xylene, hexadecanoic acid methyl ester, 3, 7, 11, 15-Tetramethyl-2-hexadecen-1-ol) which had previously reported antiproliferative and apoptotic properties. Moreover, the hexadecanoic acid methyl ester was an inhibitor of growth and apoptotic inductor of human gastric cancer cells[36]. Thus, it would be considered that MELO had the ability of an antineoplastic effect and innovative bioactive profile explored by the GC-MS study.

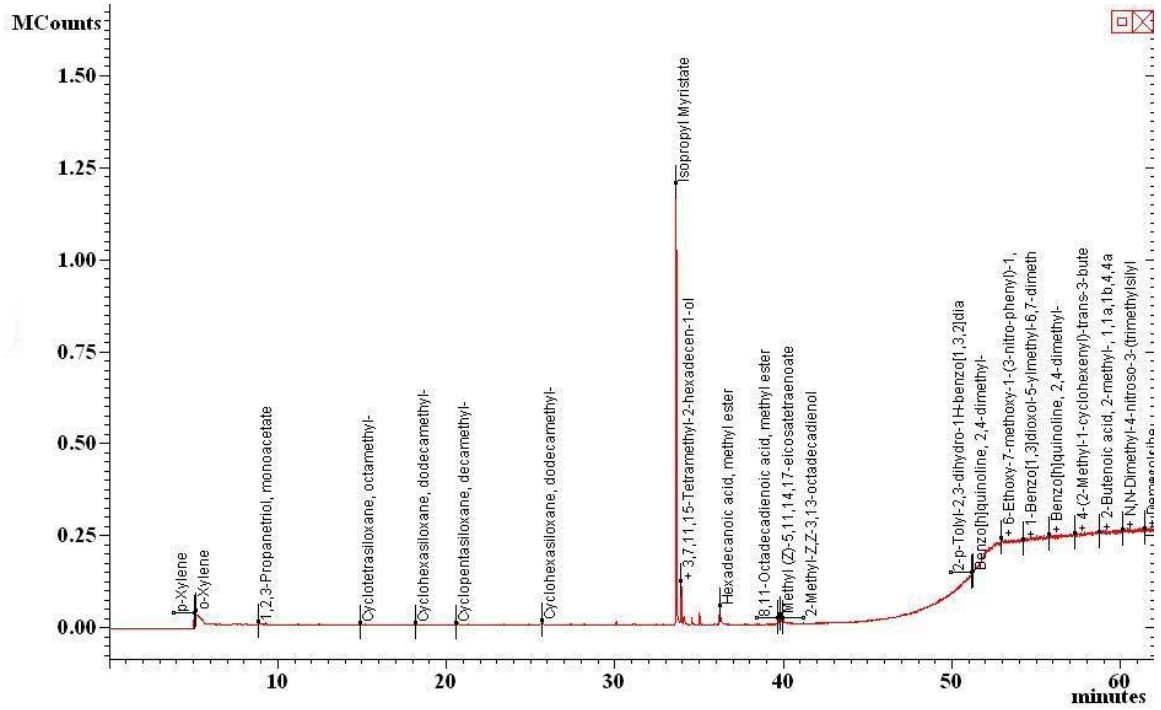
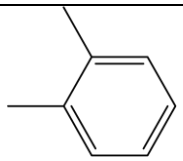
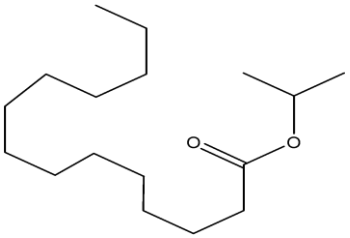
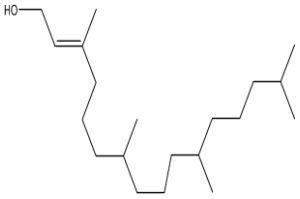
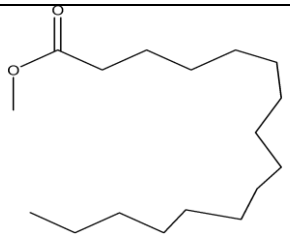
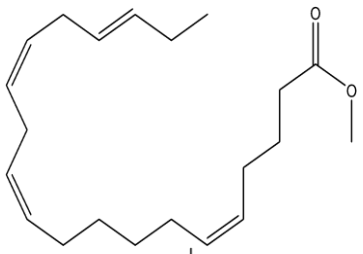
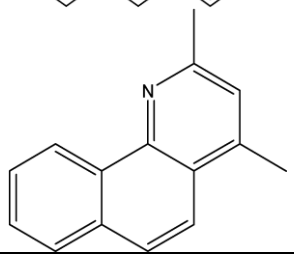


Figure 6. GC-MS chromatogram of MELO

Table 4. Chemical constituents of MELO identified by its GC-MS spectrum

Sl. No.	Name of compound	Retention time	Area	Percentage (%) composition	Name of compound
1	O-Xylene	5.10	218435	2.33	
2	Isopropyl Myristate	33.64	3782000	40.32	
3	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	33.93	532540	5.68	
4	Hexadecanoic acid, methyl ester	36.22	49319	2.66	

					
5	Methyl (Z)-5,11,14,17-eicosatetraenoate	39.79	106961	1.14	
6	2,4-dimethyl-Benzo[h]quinoline	56.76	128509	1.37	

CONCLUSION

Overall findings of this study demonstrated that the methanolic extract of *Oldenlandia corymbosa* leaves (MELO) was rich in phenols, flavonoids, flavonols and proanthocyanidins, as well as MELO showing significant antioxidant activity. This study revealed the potent inhibitory properties of MELO against EAC cells through decreasing viable EAC count and weight gain along with increasing the survival time and restoring the altered hematological parameters of cancer cell-bearing mice. In addition, the GC-MS analysis revealed the phytochemical profiles of MELO, which was also consistent with this antineoplastic activity. Thus, it can be concluded that the methanol extract of *Oldenlandia corymbosa* leaves (MELO) has the merit to consider further investigation for clarifying the mechanistic details underlying its antioxidants and antineoplastic effects.

ACKNOWLEDGMENT

The authors would like to thank A. R. M. Tareq, Environmental and Organic Laboratory, Chemistry Division, Atomic Energy Centre, Dhaka-1349, Bangladesh, for helping us take the GC-MS spectrum.

REFERENCES

1. Devasagayam T, Tilak JC, Boloor KK, Sane KS, Ghaskadbi SS, Lele RD., 2004. Free Radicals and Antioxidants in Human Health: Current Status and Future Prospects. *J Assoc Physicians India*, 52: 794-804.
2. Javanmardi J, Stushnoff C, Locke E, Vivanco JM., 2003. Antioxidant activity and total phenolic content of Iranian *Ocimum* accessions. *Food Chem*, 83: 547–50. https://doi.org/10.1016/S0308-8146(03)00151-1
3. Ozsoy N, Can A, Yanardag R, Akev N., 2008. Antioxidant activity of *Smilax*

excelsa L. leaf extracts. *Food Chem*, 110: 571–83. https://doi.org/10.1016/j.foodchem.2008.02.037

4. Islam F, Khatun H, Khatun M, Ali SMM, Khanam JA., 2014. Growth inhibition and apoptosis of Ehrlich ascites carcinoma cells by the methanol extract of *Eucalyptus camaldulensis*. *Pharm Biol*, 52: 281–90. https://doi.org/10.3109/13880209.2013.834365
5. Chen Z, Wu Q, Chen Y, He J., 2008. Effects of betulinic acid on proliferation and apoptosis in Jurkat cells and its *in vitro* mechanism. *Journal of Huazhong University of Science and Technology - Medical Science*, 28: 634–638. https://doi.org/10.1007/s11596-008-0604-9
6. Choi YH, Kong KR, Kim Y, Jung KO, Kil JH, Rhee SH, Park KY., 2003. Induction of Bax and activation of caspases during β-sitosterol-mediated apoptosis in human colon cancer cells. *International Journal of Oncology*, 23: 1657-1662. https://doi.org/10.3892/ijo.23.6.1657
7. Huang WW, Chiu YJ, Fan MJ, Lu HF, Yeh HF, Li KH, Chen PY, Chung JG, Yang JS., 2010. Kaempferol induced apoptosis via endoplasmic reticulum stress and mitochondria-dependent pathway in human osteosarcoma U-2 OS cells. *Mol Nutr Food Res*, 54: 1585–1595. https://doi.org/10.1002/mnfr.201000005
8. Das S, Mondal N, Mondal S, Ghosh P, Ghosh C, Das C, Chatterjee S, Sirshendu Chatterjee C., 2019. Botanical features, phytochemical and pharmacological overviews of *Oldenlandia corymbosa* Linn.: A brief review. *The Pharma Innovation Journal*, 8: 464–8 (2019).
9. Agrawal SK., 2013. Evaluation of antiulcer activity of *Oldenlandia corymbosa* (L). *Int J Res Dev Pharm Sci.*, 2: 363–7.
10. Patel T and JV and DR., 2014. *Oldenlandia corymbosa* L.: A Phytopharmacological review. *Int. J. Phytopharmacy*, 4: 79-82. http://dx.doi.org/10.7439/ijpp.v4i3.73
11. Archana V, Thomas NN, Lakshmi S, Rauf AA, Edwin BT., 2021. Pharmaceutical properties of *Oldenlandia corymbosa* Linn. *Materials Today: Proceedings*, 41: 698–702 (2021). https://doi.org/10.1016/j.matpr.2020.05.585
12. Rajagopal DPL, Hanima M V, Yousef S., 2018. Therapeutic utility of "*Oldenlandia corymbosa* Linn." *Journal of international academic research for multidisciplinary*, 5: 178-186
13. Nikolajsen T, Nielsen F, Rasch V, Sørensen PH, Ismail F, Kristiansen U, Jäger AK., 2011. Uterine contraction induced by Tanzanian plants used to induce abortion. *J Ethnopharmacol*, 137: 921–925 (2011). https://doi.org/10.1016/j.jep.2011.05.026
14. Kim Y, Jung Park E, Kim J, Kim Y-B, Ra Kim S, Choong Kim Y., 2000. Neuroprotective Constituents from *Hedyotis diffusa*. *J Nat Prod*, 64: 75–78. https://doi.org/10.1021/np000327d
15. Ranilla LG, Kwon YI, Apostolidis E, Shetty K., 2010. Phenolic compounds,

antioxidant activity and in vitro inhibitory potential against key enzymes relevant for hyperglycemia and hypertension of commonly used medicinal plants, herbs and spices in Latin America. *Bioresour Technol*. 2010 101(12): 4676-89. https://doi.org/10.1016/j.biortech.2010.01.093

16. Dewanto V, Wu X, K. Adom K, Hai Liu R., 2002. Thermal Processing Enhances the Nutritional Value of Tomatoes by Increasing Total Antioxidant Activity. *J Agric Food Chem*, 50: 3010–3014. https://doi.org/10.1021/jf0115589
17. Sun JS, Tsuang YH, Chen IJ, Huang WC, Hang YS, Lu FJ. 1998. An ultra-weak chemiluminescence study on oxidative stress in rabbits following acute thermal injury. *Burns*, 24: 225–231. https://doi.org/10.1016/s0305-4179(97)00115-0
18. Kumaran A, Joel Karunakaran R., 2007. *In vitro* antioxidant activities of methanol extracts of five Phyllanthus species from India. *LWT - Food Science and Technology*, 40: 344–352. https://doi.org/10.1016/j.lwt.2005.09.011
19. Prieto P, Pineda M, Aguilar M., 1999. Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum Complex: Specific Application to the Determination of Vitamin E. *Anal Biochem*, 269: 337–41. https://doi.org/10.1006/abio.1999.4019
20. Makoto Oyaizu., 1986. Studies on products of browning reaction. Antioxidative activities of products of browning reaction prepared from glucosamine. *The Japanese Journal of Nutrition and Dietetics*, 44: 307–315. https://doi.org/10.5264/eiyogakuzashi.44.307
21. Choi HY, Jhun EJ, Lim BO, Chung IM, Kyung SH, Park DK., 2000. Application of flow injection - Chemiluminescence to the study of radical scavenging activity in plants. *Phytotherapy Research*, 14: 250–3. https://doi.org/10.1002/1099-1573(200006)14:4%3C250::aid-ptr587%3E3.0.co;2-j
22. Cai Y, Luo Q, Sun M, Corke H.,2004. Antioxidant activity and phenolic compounds of 112 traditional Chinese medicinal plants associated with anticancer. *Life Sci*, 74: 2157–84. https://doi.org/10.1016/j.lfs.2003.09.047
23. Kabir SR, Zubair MA, Nurujjaman M, Haque MA, Hasan I, Islam MF, Hossain MT, Hossain MA, Rakib MA, Alam MT, Shaha RK, Hossain MT, Kimura Y, Absar N., 2011. Purification and characterization of a Ca 2+-dependent novel lectin from *Nymphaea nouchali* tuber with antiproliferative activities. *Biosci Rep*, 31: 465–475. https://doi.org/10.1042/bsr20100126
24. Khanam JA, Islam MF, Jesmin M, Ali MM. 2010. Antineoplastic activity of acetone semicarbazone (ASC) against Ehrlich ascites carcinoma (EAC) bearing mice. *Journal of the National Science Foundation of Sri Lanka*, 38: 225-231. http://doi.org/10.4038/jnsfsr.v38i4.2649
25. Ruisa V, SSK.,1988. Routine hematological tests. *In: Mukherjee, K.J. (ed.), Medical laboratory*

technology. Tata Mcgraw Hill Pub Comp Ltd, New Delhi., p.218–80.

26. Chung K-T, Wong TY, Wei C-I, Huang Y-W, Lin Y., 1998. Tannins and Human Health: A Review. *Crit Rev Food Sci Nutr*, 38: 421–464. https://doi.org/10.1080/10408699891274273
27. Dai J, Mumper RJ. 2010. Plant Phenolics: Extraction, analysis and their antioxidant and anticancer properties. *Molecules*, 15: 7313–7352. https://doi.org/10.3390/molecules15107313
28. Yang CS, Landau JM, Huang M-T, Newmark HL., 2001. Inhibition of carcinogenesis by dietary polyphenolic compounds. *Annual review of nutrition*, 21: 381-406. https://doi.org/10.1146/annurev.nutr.21.1.381
29. Gupta S, Prakash J., 2009. Studies on Indian green leafy vegetables for their antioxidant activity. *Plant Foods for Human Nutrition*, 64: 39–45. https://doi.org/10.1007/s11130-008-0096-6
30. Michael JS, Kalirajan A, Padmalatha C, Singh AJAR., 2013. *In vitro* antioxidant evaluation and total phenolics of methanolic leaf extracts of *Nyctanthes arbor-tristis* L. *Chin J Nat Med*, 11: 484–487. https://doi.org/10.1016/s1875-5364(13)60088-6
31. Segura JA, Barbero LG, Márquez J., 2000. Ehrlich ascites tumour unbalances splenic cell populations and reduces responsiveness of T cells to Staphylococcus aureus enterotoxin B stimulation. *Immunol Lett*, 74: 111–1115. https://doi.org/10.1016/s0165-2478(00)00208-x
32. Haldar PK, Kar B, Bala A, Bhattacharya S, Mazumder UK., 2010. Antitumor activity of *Sansevieria roxburghiana* rhizome against Ehrlich ascites carcinoma in mice. *Pharm Biol*, 48: 1337–1343. https://doi.org/10.3109/13880201003792592
33. Dolai N, Karmakar I, Suresh Kumar RB, Kar B, Bala A, Haldar PK., 2012. Evaluation of antitumor activity and in vivo antioxidant status of Anthocephalus cadamba on Ehrlich ascites carcinoma treated mice. *J Ethnopharmacol*, 142: 865–870. https://doi.org/10.1016/j.jep.2012.05.050
34. H.C Hogland. Hematological complications of cancer chemotherapy. *Seminal Oncology*, **9**, 95–102 (1982).
35. Jules Hirsch., 2006. An Anniversary for Cancer Chemotherapy. *The Journal of the American Medical Association*, 296: 1518–1520. https://doi.org/10.1001/jama.296.12.1518
36. Yu F-R, Lian X-Z, Guo H-Y, Mcguire PM, Li R-D, Wang R, Yu F-H, Yu F., 2005. Isolation and characterization of methyl esters and derivatives from *Euphorbia kansui* (Euphorbiaceae) and their inhibitory effects on the human SGC-7901 cells. *J Pharm Pharm Sci.*, **8**: 528-535.