



## A flavone from the ethyl acetate extract of *Leea rubra* leaves with DNA damage protection and antineoplastic activity

Nibedita Das<sup>b</sup>, Mst. Shahnaj Parvin<sup>a</sup>, Mahadi Hasan<sup>a</sup>, Masuma Akter<sup>a</sup>, Md. Sanowar Hossain<sup>c</sup>, G.M.Masud Parvez<sup>c</sup>, Ashish Kumar Sarker<sup>c</sup>, Md. Aziz Abdur Rahman<sup>a</sup>, Al Mamun<sup>a</sup>, Md. Ekramul Islam<sup>a,\*</sup>

<sup>a</sup> Department of Pharmacy, University of Rajshahi, Rajshahi, 6205, Bangladesh

<sup>b</sup> Institute of Biological Science, University of Rajshahi, Rajshahi, 6205, Bangladesh

<sup>c</sup> Department of Pharmacy, Pabna University of Science and Technology, Pabna- 6600, Bangladesh

### ARTICLE INFO

#### Keywords:

*Leea rubra*  
Oxidative DNA damage  
Antineoplastic activity  
Ehrlich ascites carcinoma

### ABSTRACT

Among the major constituents of *Leea rubra* (Family Vitaceae) leaves, phenolic and flavonoid compounds are most important for therapeutic purposes and the plant parts have been used in traditional medicine to treat several diseases for long. Thus, in order to scientifically confirm the traditional uses of the *L. rubra* leaves, the present study was designed to investigate the efficacy of the isolated flavones against AAPH induced oxidative damage to pUC19 DNA by gel electrophoresis and antineoplastic activity was evaluated on Ehrlich ascites carcinoma (EAC) bearing Swiss albino mice by evaluating percentage inhibition of cell growth, morphological changes of EAC cells and hematological parameters of the mice. The isolation was carried out by column chromatography and structure was revealed by <sup>1</sup>H-NMR and <sup>13</sup>C NMR. The result shows that, the isolated compound was identified as myricetin 4'-methoxy-3-O- $\alpha$ -L-rhamnopyranoside based on previously reported data. The isolated flavone effectively inhibited AAPH-induced oxidative damage to DNA; because it could inhibit the formation of circular and linear forms of the DNA. In anti-proliferative assay, 76% growth inhibition of EAC cells was observed as compare to the control mice ( $p < 0.05$ ) at a dose 100 mg/kg body weight. Thus the isolated flavone showed great importance as a possible therapeutic agent in preventing oxidative damage to DNA and the chronic diseases caused by such DNA damage, and can also become important in cancer chemotherapy.

### 1. Introduction

Medicinal plants have long been used for treating deadly diseases like cancer, diabetes and heart diseases because of the presence of bioactive phytochemical constituents [1]. They also have healing effects against allergic syndrome, inflammation, oxidative damage, free radical and DNA (deoxyribonucleic acid) damage [2–4]. Phytotherapy has less intense side effects than conventional synthetic drugs; and could be used as an alternative source of therapies.

Reactive oxygen species (ROS) is produced inside the human body from various chemical reactions, exogenous chemicals, endogenous metabolic processes and environmental exposition of toxic chemicals. This oxygen radicals is responsible for cellular damage by inducing lipid peroxidation of biomolecules especially lipid, protein and DNA [5]. Our body has inherent mechanism to combat against these free radicals.

Antioxidant enzymes mainly superoxide dismutase, glutathione peroxidase and catalase prevents and neutralizes these free radicals. But when excess amount of free radicals are generated inside the cell this controlling mechanisms fails and leads to the initiation of various human diseases. Excessive oxidative stress breaks double strand DNA and alters gene expression and finally mutagenesis [6].

Natural compounds have inherent potential as anticancer drugs and at present 51% of all the anticancer drugs are directly and indirectly derived from plant sources. Phenolic and flavonoids present in many plant species have cytotoxic activities against different leukemic cell lines [7–10]. Even in recent Covid-19 pandemic, the efficacy of natural products especially flavonoids is widely being investigated because of their antiviral and anti-inflammatory efficacy as well as more safety as compared to the pharmaceutical drugs [11–13].

*Leea rubra* also called Red tree shrub belongs to the Vitaceae family

\* Corresponding author.

E-mail address: [ekram@ru.ac.bd](mailto:ekram@ru.ac.bd) (Md.E. Islam).

<https://doi.org/10.1016/j.bbrep.2022.101244>

Received 10 January 2022; Received in revised form 15 February 2022; Accepted 4 March 2022

2405-5808/© 2022 The Authors.

Published by Elsevier B.V. This is an open access article under the CC BY-NC-ND license

(<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

and widely distributed in Australia, Malaysia, Thailand, Bangladesh, China and India's tropical and subtropical forest with low-medium altitude [14]. It is reported that plants belongs to the genus *Leea* possess analgesic, anti-diabetic, anticancer, anti-infective, cardio protective, wound healing, anti-diarrheal, antiulcer, anti-rheumatic, anthelmintic, antiseptic, anesthetic and anti-tubercular activities. It also has effectiveness against bronchitis, fever, itching and snake bites. But only antimicrobial and anti-inflammatory activities were reported from *L. rubra* in recent study [15,16].

It is established that *L. rubra* has medicinal potentials and our objective is to isolate pure compound from the leaves and determine its biological properties. So the rationale of his study was to isolate and characterize compounds from the ethyl acetate leaf extract of *L. rubra* and to evaluate antineoplastic activity against EAC cell lines and to confirm the protecting activity on DNA from oxidative damage.

## 2. Materials and methods

### 2.1. Chemicals and reagents

2'-azobis (2-methylpropionamide) dihydrochloride (AAPH), agarose, ethidium bromide, bovine serum albumin, gallic acid, catechin, homovanillic acid, epicatechin, chlorogenic acid, rutin hydrate and quercetin-3-rhamnoside were purchased from Sigma-Aldrich, St. Louis, MO, USA. pUC19 plasmid DNA was purchased from Genetix, Bangalore, India. The other chemicals utilized in the tests were of analytical grade and purchased from the Sigma-Aldrich and Roche.

### 2.2. Plant material and preparation of the extract

In September 2016, fresh leaves of *Leea rubra* were collected from forests in Bandarban district, Bangladesh. This plant specimen was authenticated in Botany Department by a taxonomist of University of Rajshahi. The leaves were preserved in Phytochemistry laboratory in Pharmacy Department, University of Rajshahi, Bangladesh for further investigation.

At first the fresh leaves were washed thoroughly by distilled water to remove unwanted particles, dirt's and parasites. Then the leaves were shed dried, crushed into coarse powder by grinding machine, and extracted by methanol and fractionated sequentially with n-hexane, chloroform and ethyl acetate. After that chloroform (CF) and ethyl acetate extract (EAF) was filtered through Whatman® No.1 filter paper and n-hexane extract was discarded. The collected extracts were concentrated under vacuum on a rotary evaporator (Heidolphrotacool, Germany) to get thick residue which was used for further study.

### 2.3. Isolation and characterization of the compound

The extract EAF had potential activity compared to the rest of the fractions and TLC assay also indicated some distinct and prominent spot on EAF extract. So that EAF extract was focused for further purification and separation of bioactive compounds. This extract was fractionated in a silica gel 60 column chromatography by using n-hexane as mobile phase. The column was run by n-hexane with increasing percentages of ethyl acetate and total 304 fractions were collected to identify pure compound. The fractions which have same spot on TLC plate were joined together and purified by preparative thin layer chromatography (PTLC) to separate and identify the target compound. Among the isolated fractions, compound **Sz 04** was seems to be pure. Then the structure of the **Sz 04** was interpreted by <sup>1</sup>H and <sup>13</sup>C NMR (Nuclear Magnetic Resonance) spectra on Jeol-Ex at 400 MHz and 100 MHz; and Fourier-transform infra-red spectroscopy (FTIR). The structure was compared with the reported compounds which were found on literature [17–19].

### 2.4. Protective effect on oxidative DNA damage

DNA damage index was determined from transformation of super-coiled plasmid DNA (S-form) to open circular DNA (C-form) following the formation of linear DNA (L-form). Plasmid DNA (pUC19) was used for assessing the protective activity of the EAF extract against DNA damage [20]. At first 10 µg of pUC19 plasmid was added to the different concentrations of the sample with gallic acid as standard compound. Subsequently, 2 µl of 25 mM AAPH in PBS (pH 7.4) was added to the mixture. The resulting mixture was incubated at 37°C for 30 min, electrophoresed on agarose gel (2%) which contains ethidium bromide at 0.5 µg/ml concentration. Finally intensities of different band were analyzed by using gel visualization system and the band intensity was measured by using GelAnalyzer software (v. 19.1).

### 2.5. Antineoplastic activity

The Ehrlich's ascites carcinoma (EAC) cell lines (1 × 10<sup>6</sup> cells/mouse) were used to determine tumor cell growth inhibition properties. The tumor cell were incubated on four group (Group-I, II, III & IV) of Swiss albino mice (n = 4) at day zero. After 24 h of inoculation, treatments were started and continued for the next 7 days. Mouse of group-II and Group-III were treated with the isolated pure compound **Sz 04** at 25 mg/kg and 50 mg/kg body weight daily dose, and mouse of Group-IV were treated by bleomycin (Standard anticancer drug) at 3 mg/kg body weight. Group-I mouse were regarded as control group and did not received any intervention. The animals were sacrificed on day 7 and tumor cells (EAC) were collected by repeatedly washing the intraperitoneal layer with 0.9% saline solution (NaCl). The number of viable tumor cells (EAC) per mouse of the Group-II and Group-III were compared with the control group [21].

#### 2.5.1. Experimental tumor and animal model

The transplantable tumor cell line (EAC) was collected from Department of Biochemistry, University of Rajshahi and cultured in Pharmacy Department, Rajshahi University. Intra peritoneal route was used to transplant the tumor cell to the Swiss albino mice. All mice used in the experiment were collected from Jahangirnagar University animal breeding centre and all were male mice, hence no variation in effect due to gender was observed.

#### 2.5.2. Determination of median lethal dose (LD<sub>50</sub>)

The isolated pure compound was injected intraperitoneally in mice and determined Median lethal dose (LD<sub>50</sub>) from the curve of mortality against at various doses (5, 10, 25, 50, 100 and 200 mg/kg). At the end of 24 h of experiment morality was recorded [22].

#### 2.5.3. Morphological appearance of EAC cell of control mice and treated mice

After collecting the cells from treated (by pure compound 25 and 50 mg/kg/day) and non-treated EAC bearing mice, morphological changes of EAC cells were investigated by DAPI (4, 6-diamidino-2-phenylindole) staining. Visual image was recorded by fluorescent microscope and both optical and fluorescent images were observed.

#### 2.5.4. Ethics statement

Approval of animal studies was taken from the ethical committee of Institute of Biological Sciences, University of Rajshahi, Bangladesh in compliance with the guide for the care and handling of animals in the laboratory. Institutional Animal, Medical Ethics, Bio-Safety and Bio-Security Committee (IAMEBBC) for Experimentations on Animal, Human, Microbes, and Living Natural Sources at University of Rajshahi approved the trial protocol for using human blood cells (reference number: 31/320-IAMEBBC/IBSc).

## 2.6. Statistical analysis

One-way ANOVA (analysis of variance) was used to analyze the experimental data followed by multiple comparisons with Dunnett's post hoc test by SPSS (Statistical Package for the Social Sciences) software, version 16. The results were represented as mean  $\pm$  standard deviation (SD). P value  $< 0.05$  was considered to determine the significance of the experimental data.

## 3. Results

### 3.1. Spectral analysis of the isolate

The presence of a flavonoid nucleus in the compound (**Sz 04**) was indicated from IR spectrum (KBr) of the compound (**Sz 04**) which showed peaks at 3434 (OH), 2926 (-CH-), 1628 (C=O), 1460 (-C=C-), 1385, 1291, (-C-O- bend), 1193, 1151 (-C-O- stretch), 765, 657, and 602 (-C-H out of plane bending)  $\text{cm}^{-1}$ . In addition, characteristic features of a flavone nucleus with glycosidic bond also revealed from  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectral data of the isolate [23]. Fig. 1 denotes the  $^1\text{H}$ -NMR spectrum of the compound (**Sz 04**). A distinct meta-coupled doublet (each  $J = 2.0$  Hz), one at  $\delta$  6.36 (C-6) and the other at 6.59 (H, d, 2H, H-8) observed from spectrum analysis which is characteristic features of protons on A ring of flavone nucleus. Moreover, presence of protons in B ring or flavone nucleus indicated by one singlet of two protons at  $\delta$  6.90.  $^{13}\text{C}$  NMR (Fig. 2) data showed 22 carbon signals and a typical flavonol  $\delta$ -L-rhamnopyranoside pattern with characteristic sugar unit comprising six carbons where  $\delta$  103.7 (C-1'') is an anomeric carbon and  $\delta$  17.8 (C-6'') is a methyl carbon of rhamnose unit. All the data established the existence of 4'-methoxy myricetin structure and O-rhamnopyranoside linkage at C-3 position (137.0). The findings were very similar and in good agreement with some of the previous research study and data [24–26]. Therefore, compound (**Sz 04**) was identified as myricetin 4'-methoxy-3-O- $\alpha$ -L-rhamnopyranoside (Mearnsitrin, Fig. 3).

### 3.2. DNA damage protection

The protecting effect of the natural compounds against oxidative damage to the cellular components is widely studied on supercoiled plasmid DNA with some well established method. The ability of the compounds to prevent the conversion of supercoiled form (S-form) of plasmid DNA to circular (C-form) and linear form (L-form) due to free radical damage is measured as a parameter of protection.

### 3.2.1. Protection against oxidative DNA damage

In this study, the ability of the isolated compound (flavone) to protect DNA damage due to oxidation was assessed by studying the band pattern of pUC19 DNA on agarose gel as shown in Fig. 4. The native super-coiled form of DNA was observed in Lane-1, whereas in lane-2 which was treated with AAPH, the super coil form has been transformed into open circular DNA. The transformation of super coil form into circular form of plasmid DNA was prevented by the test compound in a dose dependent manner, which was observed after addition of the compound in lane 3, 4 and 5 at concentration of 20, 30 and 40  $\mu\text{g}/\text{ml}$  respectively. Here, gallic acid was used as standard at a dose 40  $\mu\text{g}/\text{ml}$ . The intensity of different bands for S-form, C-form and L-form of plasmid DNA has been shown in fig. 4 (a & b).

### 3.3. Antineoplastic activity

Antineoplastic activity of the isolated flavones was determined by measuring its capability to prevent EAC cell growth, promote apoptosis and restoration of normal hematological parameters in the mice model.

#### 3.3.1. Studies on EAC cell growth inhibition

Ability of a compound to prevent the growth of cancer cells in a well established model like EAC cell is the major indicator to prove the antineoplastic activity of the compound. So, the antineoplastic activity of the isolated flavone (myricetin 4'-methoxy-3-O- $\alpha$ -L-rhamnopyranoside) was evaluated by the % of inhibition in cell growth against EAC cell bearing mice and is shown in Table 1.  $64.53 \pm 4.73\%$  and  $37.02 \pm 6.92\%$  cell growth inhibition was observed with pure compound at doses of 50 and 25 mg/kg respectively. The anticancer drug bleomycin showed  $85 \pm 5.2\%$  inhibition of cell growth at 3 mg/kg. The result designates significant ( $P < 0.05$ ) decrease in cell growth *in vivo* compared with the control mice.

#### 3.3.2. Apoptosis assay

Promoting apoptosis can inhibit the growth of cancer cells by damaging them. So, damaged cellular components in the cancer cells after treating with the target compound can prove its ability to promote apoptosis and effectiveness against cancer. In this study, morphological changes of EAC cells from non-treated EAC-bearing mice and mice treated with the compound were examined by DAPI staining. EAC cells of normal group mouse were round, regular with normal shaped nucleus under fluorescence microscope, whereas in compound treated cells, irregular, fragmented and condensed nucleus was observed.

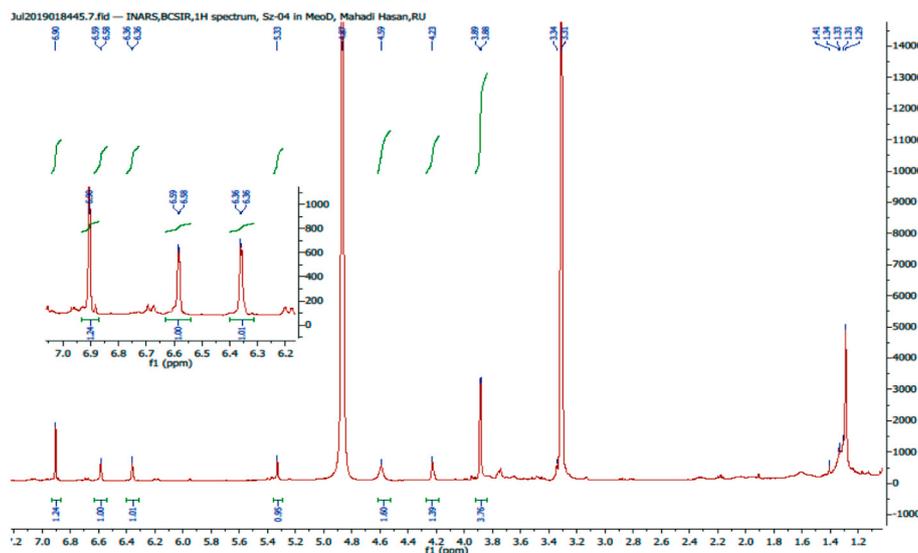


Fig. 1. Proton NMR spectrum of the compound Sz 04.

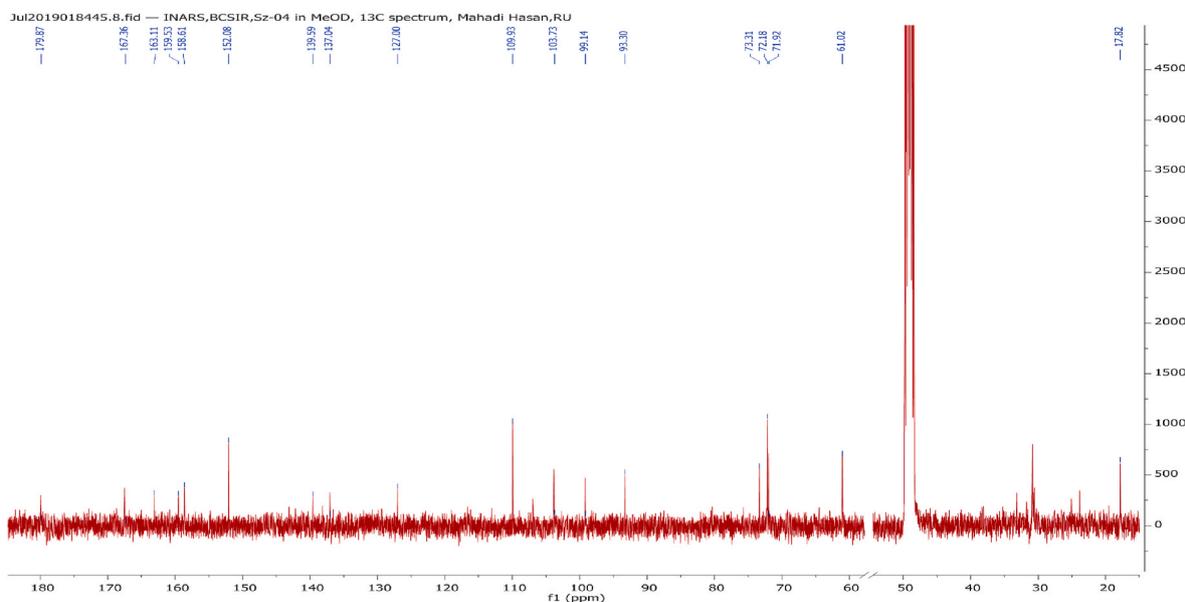


Fig. 2. Carbon NMR spectrum of the compound Sz 04.

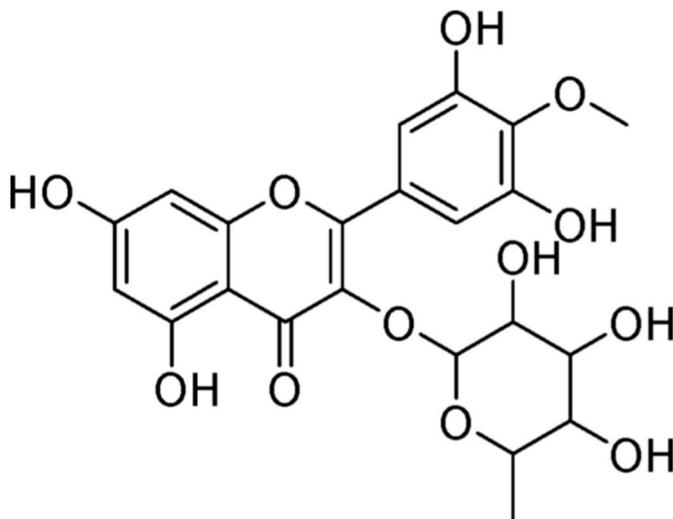


Fig. 3. Chemical structure of the compound Sz-04.

Fluorescence and optical microscopic view of EAC cells of treated and control mice are presented in Fig. 5.

### 3.3.3. Studies on hematological parameters

Alteration of the hematological parameters i.e.-decreased RBC and hemoglobin count and increased WBC count is a common morphological change in cancer patient. So, the hematological parameters of the untreated EAC cell bearing mice (negative control) were measured on day 12, where hematological parameters exhibited substantial changes in comparison with normal mice ( $P < 0.05$ ) and the result is shown in Table 2. Both hemoglobin content and total RBC count were decreased but total WBC count was increased. Treatment with the compound (25 and 50 mg/kg) could bring back these altered parameters to normal values at the same time interval.

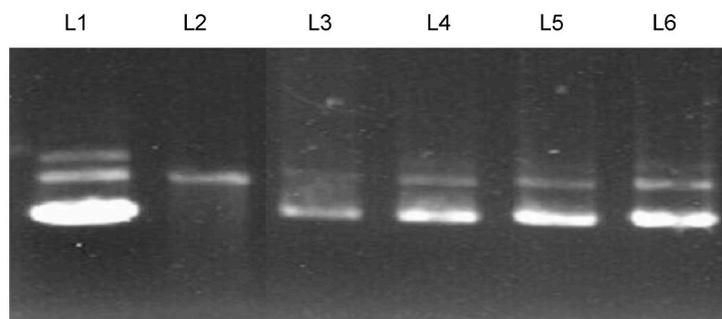
The overall results of the above experiment clearly established the significant protective activity of the isolated flavone against free radical induced damage to cellular components (DNA) and also exhibited potential antineoplastic activity against EAC cell growth and reduction of the morphological changes of normal blood cells.

## 4. Discussion

Plant extracts contain naturally occurring many complex chemical compounds which are responsible for the biological activity of the extracts and can act alone or synergistically. Previous phytochemical studies with the leaves of *Leea* genus showed an abundance of phenolic constituents such as flavonoids, leucoanthocyanidins, p-hydroxybenzoic acid, syringic acid and gallic acid [27]. In our study, myricetin 4'-methoxy-3-O- $\alpha$ -L-rhamnopyranoside, commonly known as meansitrin was isolated from leaves extract of *L. rubra* and this the first time reported isolated compound from this plant. Spectral data of the isolate revealed characteristic features of a flavone nucleus with glycosidic bond and is structurally very similar to myricetin which is a common plant-derived flavonoid. This compound exhibits a wide range of activities including antioxidant activity, antineoplastic activity, anti-diabetic activity and anti-inflammatory activity. The flavonoid compounds also displayed several other activities that are related to the central nervous system and numerous studies have suggested that the compound may be beneficial to protect against diseases such as Parkinson's and Alzheimer's disease [28,29]. To know the more biological activity of the isolated compound, we determined *in vitro* protection of oxidative damage on DNA as well as anti-proliferative activity on EAC cells. The results of these experiments showed promising results.

Cellular DNA damage may cause alteration of replication and transcription of DNA and can result in to cell death or mutations. Such modification of DNA is responsible for aging and various diseases, including Alzheimer, Parkinson and cancer etc. The use of antioxidants to protect DNA damage can be beneficial for suppressing oxidative damage to DNA and thus potentially preventing such chronic human diseases [30,31]. So, study on DNA damage protection is important to justify the activity of the natural compounds against the chronic diseases or accelerated ageing. Here we found the effectiveness of the isolated flavone to prevent DNA damage induced by AAPH. Free radical from AAPH reacts with DNA bases generating sugar and base radicals causing the breakdown of sugar-phosphate backbone resulting in strand breaks and formation of open circular DNA from super coiled DNA [32]. A dose-dependent DNA damage protective activity was observed with the sample. The antioxidant capacity of myricetin 4'-methoxy-3-O- $\alpha$ -L-rhamnopyranoside at a concentration of 40  $\mu$ g/ml could manage oxidative damage protection by inhibiting the excess of free radicals. Our results are also in line with other authors where, K. Soumya

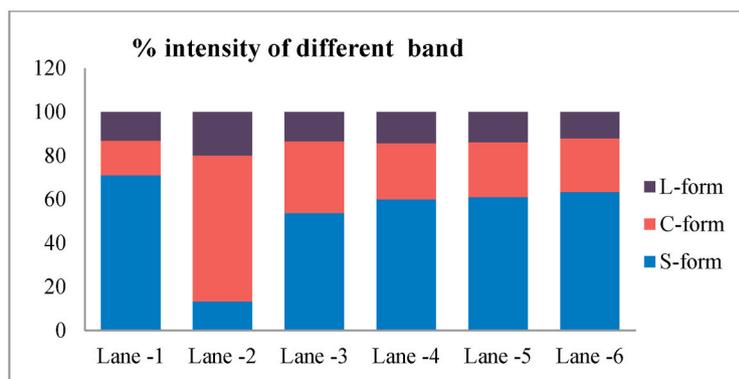
a.



L-form  
C-form  
S-form

**Fig. 4.** (a & b). Protective effect of the isolated compound on DNA damage induced by AAPH. Lane-1: Native DNA, Lane-2: DNA + AAPH, Lane-3: DNA + AAPH + 20 µg of compound B, Lane-4: DNA + AAPH + 30 µg of compound B, Lane-5: DNA + AAPH + 40 µg of compound B, Lane-6: DNA + AAPH + 40 µg of gallic acid. Each lane contains 10 µg DNA. Here, S-form is the super coiled form of plasmid DNA, C-form is the open circular form of plasmid DNA and L-form is the linear form of plasmid DNA.

b.

**Table 1**

Effect of compound on viable Ehrlich ascites carcinoma (EAC) cell growth.

Treatment Group	Dose mg/kg/day	No of viable EAC cells on day 7 after inoculation ( $\times 10^7$ cells/ml)	% of Cell growth inhibition
Control	-	61.14 $\pm$ 2.23	-
Compound	25 mg/kg	38.97 $\pm$ 4.23*	37.02 $\pm$ 6.92*
	50 mg/kg	22.778 $\pm$ 2.89*	64.53 $\pm$ 4.73*
Bleomycin	3 mg/kg	13.78 $\pm$ 1.58	85 $\pm$ 5.2

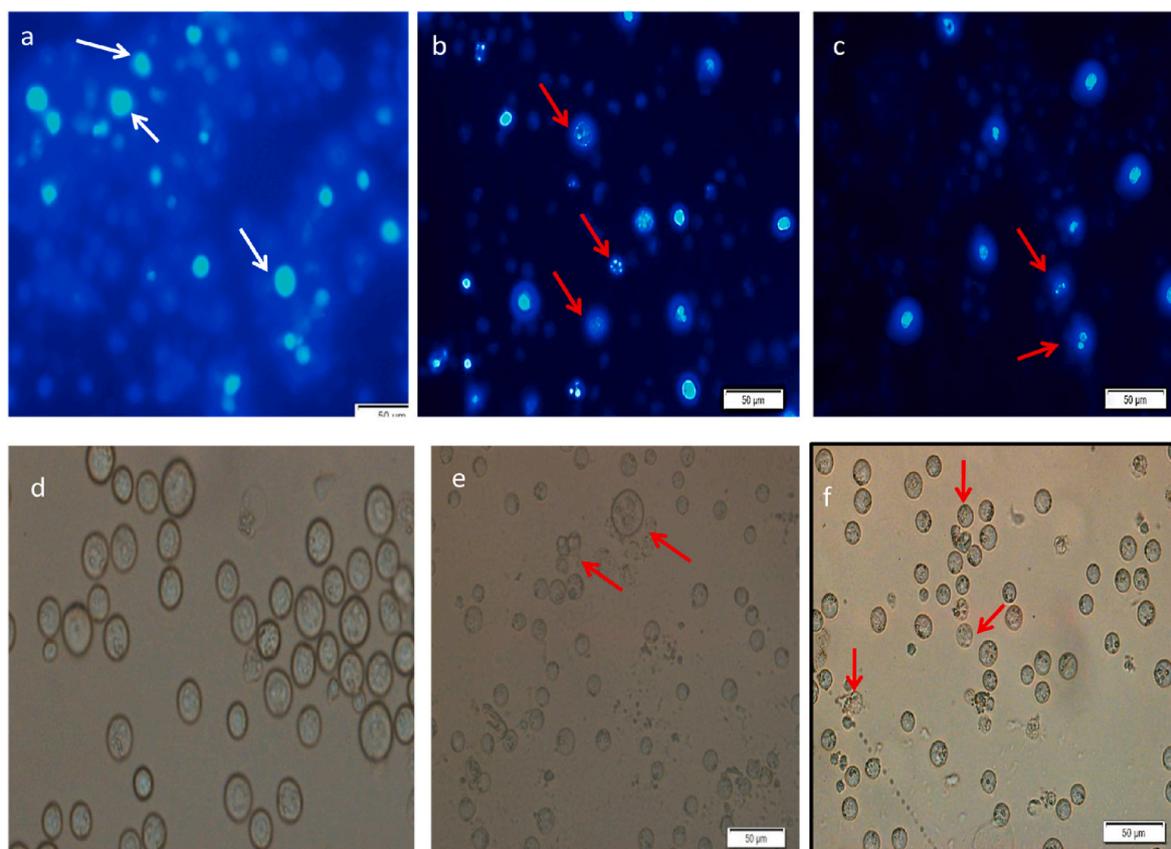
Data are expressed as mean  $\pm$  SD (n = 5); Analysis of variance followed by LSD and Dunnett's post hoc test (IBM-SPSS/20) \*P<0.05: Significance difference with respect to EAC control.

et al. reported the efficacy of flavones (luteolin) isolated from the fruit of *Terminalia chebula* against oxidative damage of DNA [33] and reported that the flavone significantly inhibited the formation of circular and linear forms of plasmid DNA. Even supercoiled percentage of pUC19 plasmid DNA observed for treatment with the pure compound in our study is almost higher than other standard antioxidants like catechin, luteolin, apigenin, quercetin and Kaempferol [34]. In addition, it also showed similar DNA damage protective activity compared to standard gallic acid at the same concentration (Fig. 4 a & b). Binding of the phenolic compound to DNA directly with the phosphate backbone is considered as the probable mechanisms of protection to DNA [35,36]. The compound could significantly inhibit the formation of the open circular form in a dose dependent manner as compared to standard gallic acid. Evidences from literature tell us flavonoids of plants can reduce DNA damage through inhibition of reactive oxygen species [37]. The formation of complexes with DNA by the flavonoids to protect the DNA from oxidative damage has been reported by scientific studies [38]. Since strand breakage of DNA can lead to mutagenesis cytotoxicity and carcinogenesis [39], this result demonstrated the DNA damage inhibition potential of this compound, therefore, can be used in cancer prevention.

So, next we investigated the role of this compound in cell growth inhibition by *in vivo* study on EAC cell bearing Swiss albino mice and it was able to inhibit the growth of EAC cell significantly. The EAC cells were used as experimental tumor models in the study because of the well known and wide use of the model in cancer research [40].

To evaluate the cell damaging capability of the isolated compound, the neoplastic cells collected from the flavone treated mice group were observed and the cells showed some structural changes with damage in the inner cell membrane. The cells were also evident with fragmented nucleus, condensed chromosome and shrinkage under fluorescence microscope. On the other hand, EAC cells collected from control mice were normal in appearance (Fig. 5). Thus, it can be said that the compound was able to damage and prevent growth of the tumor cells because of the morphological changes in cells. This activity may be due to the enhanced apoptosis in the mice, because lack of apoptosis can lead to abnormal cell proliferation and cancer development. The change in morphological and biological parameters like reduction in RBC or % in hemoglobin and increased WBC count is common during cancer chemotherapy because of the deficiency of iron or hemolysis due to the therapy [41]. Similar condition was found in our experimental animals and the hematological parameters were restored to near normal values by the therapy with significant recovery of the hemoglobin content; RBC and WBC count; that indicate the protective action of the compound on haemopoietic system during cancer chemotherapy. Thus, it can be said that the isolated flavones from *L. rubra* possess significant antineoplastic activity as well as protective activity against chemotherapy induced damage to blood cells.

So the study was clinically very significant and indicated a great clinical value of the isolated flavone especially in treating cancer and other chronic diseases caused by free radical induced damage to the cellular components. Plant extract containing flavonoid derivatives has shown such activities by most of the related scientific studies [28]. So the assumption for a significant anticancer and DNA damage protecting activity by the isolated flavones has been proved scientifically by the



**Fig. 5.** (a–c) Fluorescence and (d–f) optical microscopic observation of EAC cells for control mice and treated mice. (a) Represents fluorescence microscopic view of control and (b–c) pure compound treated mice cell whereas (d) express the optical microscopic view of control and (e–f) pure compound treated cells. Normal cell with round shape nuclei appeared in control group indicated by white arrow in (a); whereas the mice treated with pure compound, condensed nucleus and fragmentation of cells (apoptotic characteristics) were found in (b) and (c) indicated by white and in (e) and (f) by red arrow, respectively. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

**Table 2**

Effect of pure compound on blood parameters of tumor bearing and normal mice.

Parameter	Normal Mice	EAC+ Control	EAC+ Compound 25 mg/kg	EAC+ Compound 50 mg/kg	EAC+ Belomycin 3 mg/kg
Hgb(g/dl)	13.89 ± 0.08	8.41 ± 0.19*	10.51 ± 0.37 <sup>#</sup>	11.66 ± 0.17 <sup>#</sup>	12.47 ± 0.19 <sup>#</sup>
RBC (x10 <sup>9</sup> cells/ml)	6.15 ± 0.094	2.21 ± 0.168*	3.83 ± 0.165 <sup>#</sup>	4.35 ± 0.165 <sup>#</sup>	5.67 ± 0.156 <sup>#</sup>
WBC (x10 <sup>9</sup> cells/ml)	8.176 ± 1.12	120.7 ± 1.81*	64.37 ± 0.92 <sup>#</sup>	36.81 ± 2.193 <sup>#</sup>	26.38 ± 1.92 <sup>#</sup>

Data are expressed as mean ± SD (n = 5); Analysis of variance followed by LSD and Dunnett's post hoc test (IBM-SPSS/20); \*P<0.05: against normal group and <sup>#</sup>P<0.05: against EAC control group.

study; and the study also indicated the necessity of further investigation on the compound to design and develop an effective drug that could be a safe alternative to many of the currently available anticancer drugs with remarkable side effects [42]. Along with the above effect, different studies have reported significant activity of flavonoid derivatives on central nervous system, psychiatric disorder, digestive function, neuroprotection, antimicrobial activity etc. [43,44]. So, further investigations can be made to evaluate such effects of the isolated flavones as source of potential therapy.

## 5. Conclusion

The overall Findings of the study shows that, the effectiveness of the

isolated compound myricetin 4'-methoxy-3-O- $\alpha$ -L-rhamnopyranoside from the leaves of *L. rubra* to prevent oxidative damage of DNA has been found to be very significant. At the same time, its effect on the inhibition of tumor cell growth and restoring the hematological parameters was notable and can be a potential candidate for further investigation for antineoplastic drugs.

## Funding

This study was partially supported by Scientific Research Projects Grant of Rajshahi University (Project Number: 5/52/RU/Sci.-31/2017–2018) to carry out the experiment.

## Author's contribution

EI designed and supervised the study; ND conducted the experiments; SP, SH, MP and AKS prepared and revised the manuscript; AR analyzed the spectral characteristics of the compound and MA, SH and MH helped in conducting the experiments. All the authors read and approved the manuscript.

## Declaration of competing interest

The authors declare that there are no conflicts of interest.

## Data availability

Data will be made available on request.

## Acknowledgement

The author is thankful to the Institute of Biological Science (IBSc), University of Rajshahi for its support for the study and Department of Biochemistry and Molecular Biology, University of Rajshahi for providing EAC cells.

## References

- [1] I. Chatti, I. Limem, J. Boubaker, I. Skandrani, S. Kilani, W. Bhouri, et al., Phytochemical, antibacterial, antiproliferative, and antioxidant potentials and DNA damage-protecting activity of *Acacia salicina* extracts, *J. Med. Food* 12 (3) (2009) 675–683, <https://doi.org/10.1089/jmf.2008.0120>.
- [2] M. Uddin Chy, M. Adnan, M. Chowdhury, E. Pagano, A. Kamal, K. Oh, et al., Central and peripheral pain intervention by *Ophiorrhiza rugosa* leaves: potential underlying mechanisms and insight into the role of pain modulators, *J. Ethnopharmacol.* 276 (2021) 114182, <https://doi.org/10.1016/j.jep.2021.114182>.
- [3] M. Bari, L. Khandokar, E. Haque, B. Romano, R. Capasso, V. Seidel, et al., Ethnomedicinal uses, phytochemistry, and biological activities of plants of the genus *Gynura*, *J. Ethnopharmacol.* 271 (2021) 113834, <https://doi.org/10.1016/j.jep.2021.113834>.
- [4] L. Čipák, P. Rauko, E. Miadoková, I. Čipáková, L. Novotný, Effects of flavonoids on cisplatin-induced apoptosis of HL-60 and L1210 leukemia cells, *Leuk. Res.* 27 (1) (2003) 65–72, [https://doi.org/10.1016/s0145-2126\(02\)00063-2](https://doi.org/10.1016/s0145-2126(02)00063-2).
- [5] V. Lobo, A. Patil, A. Phatak, N. Chandra, Free radicals, antioxidants and functional foods: impact on human health, *Phcog. Rev.* 4 (8) (2010) 118, <https://doi.org/10.4103/0973-7847.70902>.
- [6] N. Degtyareva, L. Heyburn, J. Sterling, M. Resnick, D. Gordenin, P. Doetsch, Oxidative stress-induced mutagenesis in single-strand DNA occurs primarily at cytosines and is DNA polymerase zeta-dependent only for adenines and guanines, *Nucleic Acids Res.* 41 (19) (2013) 8995–9005, <https://doi.org/10.1093/nar/gkt671>.
- [7] D. Newman, G. Cragg, Natural products as sources of new drugs over the 30 Years from 1981 to 2010, *J. Nat. Prod.* 75 (3) (2012) 311–335, <https://doi.org/10.1021/np200906s>.
- [8] J. Fernández, B. Silván, R. Entrialgo-Cadierno, C. Villar, R. Capasso, J. Uranga, et al., Antiproliferative and palliative activity of flavonoids in colorectal cancer, *Biomed. Pharmacother.* 143 (2021) 112241, <https://doi.org/10.1016/j.biopha.2021.112241>.
- [9] K. Sinan, U. Akpulat, A. Aldahish, Y. Celik Altunoglu, M. Baloğlu, D. Zheleva-Dimitrova, et al., LC-MS/HRMS analysis, anti-cancer, anti-enzymatic and antioxidant effects of *Boerhavia diffusa* extracts: a potential raw material for functional applications, 2003, *Antioxidants* 10 (12) (2021), <https://doi.org/10.3390/antiox10122003>.
- [10] M. Rahman, A. Reza, M. Khan, K. Sujon, R. Sharmin, M. Rashid, et al., Unfolding the apoptotic mechanism of antioxidant enriched-leaves of *Tabebuia pallida* (lindl.) miers in EAC cells and mouse model, *J. Ethnopharmacol.* 278 (2021) 114297, <https://doi.org/10.1016/j.jep.2021.114297>.
- [11] T. Tallei, Fatimawali, N. Niode, R. Idroes, B. Zidan, S. Mitra, et al., A comprehensive review of the potential use of green tea polyphenols in the management of COVID-19, *Evid. base Compl. Alternative Med.* 2021 (2021) 1–13, <https://doi.org/10.1155/2021/7170736>.
- [12] D. Ağaçgündüz, M. Çelik, M. Çıtar Dazıroğlu, R. Capasso, Emergent drug and nutrition interactions in COVID-19: a comprehensive narrative review, *Nutrients* 13 (5) (2021) 1550, <https://doi.org/10.3390/nu13051550>.
- [13] S. Mousavi, A. Karami, T. Haghighi, S. Tumilaar, Fatimawali, R. Idroes, et al., In silico evaluation of Iranian medicinal plant phytoconstituents as inhibitors against main protease and the receptor-binding domain of SARS-CoV-2, *Molecules* 26 (18) (2021) 5724, <https://doi.org/10.3390/molecules26185724>.
- [14] N. Reddy, S. Navanesan, S. Sinniah, N. Wahab, K. Sim, Phenolic content, antioxidant effect and cytotoxic activity of *Lea indica* leaves, *BMC Compl. Alternative Med.* 12 (1) (2012), <https://doi.org/10.1186/1472-6882-12-128>.
- [15] N. Das, S. Hossain, J. Barmon, S. Parvin, M. Hasan, M. Akter, E. Islam, Evaluation of *leea rubra* leaf extract for oxidative damage protection and antitumor and antimicrobial potential, *J. Trop. Med.* 2021 (2021) 1–8, <https://doi.org/10.1155/2021/7239291>.
- [16] M. Ongsakul, A. Jindarat, C. Rojanaworarit, Antibacterial effect of crude alcoholic and aqueous extracts of six medicinal plants against *Staphylococcus aureus* and *Escherichia coli*, *J. Health Res.* 23 (3) (2018) 153–156. Retrieved from, <https://h.e01.tci-thaijo.org/index.php/jhealthres/article/view/156513>.
- [17] V. Seidel, F. Baillleul, P. Waterman, Rel)-1β,2α-di-(2,4-dihydroxy-6-methoxybenzoyl)-3β, 4α-di-(4-methoxyphenyl)-cyclobutane and other flavonoids from the aerial parts of *Goniothalamus gardneri* and *Goniothalamus thwaitesii*, *Phytochemistry* 55 (5) (2000) 439–446, [https://doi.org/10.1016/s0031-9422\(00\)00346-0](https://doi.org/10.1016/s0031-9422(00)00346-0).
- [18] R. Xu, X. Yang, J. Wang, H. Zhao, W. Lu, J. Cui, et al., Chemical composition and antioxidant activities of three polysaccharide fractions from pine cones, *Int. J. Mol. Sci.* 13 (12) (2012) 14262–14277, <https://doi.org/10.3390/ijms131114262>.
- [19] S. Kumar, A. Pandey, Chemistry and biological activities of flavonoids: an overview, 2013, *Sci. World J.* (2013) 1–16, <https://doi.org/10.1155/2013/162750>.
- [20] F. Zhao, Z. Liu, The protective effect of hydroxyl-substituted Schiff bases on the radical-induced oxidation of DNA, *J. Phys. Org. Chem.* 22 (9) (2009) 791–798, <https://doi.org/10.1002/poc.1517>.
- [21] F. Islam, H. Khatun, S. Ghosh, M. Ali, J. Khanam, Bioassay of Eucalyptus extracts for anticancer activity against Ehrlich ascites carcinoma (eac) cells in Swiss albino mice, *Asian.Pacific J. Trop. Biomed.* 2 (5) (2012) 394–398, [https://doi.org/10.1016/s2221-1691\(12\)60063-x](https://doi.org/10.1016/s2221-1691(12)60063-x).
- [22] J.T. Litchfield Jr., F. Wilcoxon, A simplified method of evaluating dose-effect experiments, *J. Pharmacol. Exp. Therapeut.* 96 (2) (1949) 99–113.
- [23] M. Henczowski, M. Kopacz, D. Nowak, A. Kuźniar, Infrared spectrum analysis of some flavonoids, *Acta Pol. Pharm.* 58 (6) (2001) 415–420.
- [24] A. El Antri, N. Lachkar, H. El Hajjaji, F. Gaamoussi, B. Lyoussi, B. El Bali, et al., Structure elucidation and vasodilator activity of methoxy flavonols from *Calycotome villosa* subsp. *intermedia*, *Arab. J. Chem.* 3 (3) (2010) 173–178, <https://doi.org/10.1016/j.arabjc.2010.04.006>.
- [25] Myricetin, Retrieved from, <https://pubchem.ncbi.nlm.nih.gov/compound/Myricetin>, 2022. (Accessed 13 November 2022).
- [26] A. Youssef Moustafa, A. Khodair, M. Saleh, Isolation, structural elucidation of flavonoid constituents from *Leptadenia pyrotechnica* and evaluation of their toxicity and antitumor activity, *Pharmaceut. Biol.* 47 (6) (2009) 539–552, <https://doi.org/10.1080/13880200902875065>.
- [27] F. Hossain, M. Mostofa, A. Alam, Traditional uses and pharmacological activities of the genus *leea* and its phytochemicals: a review, *Heliyon* 7 (2) (2021), e06222, <https://doi.org/10.1016/j.heliyon.2021.e06222>.
- [28] D. Semwal, R. Semwal, S. Combrinck, A. Viljoen, Myricetin: a dietary molecule with diverse biological activities, *Nutrients* 8 (2) (2016) 90, <https://doi.org/10.3390/nu8020090>.
- [29] M. Freitas, A. Vasconcelos, E. Gonçalves, E. Ferrarini, G. Vieira, D. Cicia, et al., Involvement of opioid system and TRPM8/TRPA1 channels in the antinociceptive effect of spirulina platensis, *Biomolecules* 11 (4) (2021) 592, <https://doi.org/10.3390/biom11040592>.
- [30] D. Praticò, N. Delanty, Oxidative injury in diseases of the central nervous system: focus on alzheimer's disease, *Am. J. Med.* 109 (7) (2000) 577–585, [https://doi.org/10.1016/s0002-9343\(00\)00547-7](https://doi.org/10.1016/s0002-9343(00)00547-7).
- [31] S. Ahmed, H. Khan, M. Aschner, H. Mirzae, E. Küpeli Akkol, R. Capasso, Anticancer potential of furanocoumarins: mechanistic and therapeutic aspects, *Int. J. Mol. Sci.* 21 (16) (2020) 5622, <https://doi.org/10.3390/ijms21165622>.
- [32] Y. Yoshioka, X. Li, T. Zhang, T. Mitani, M. Yasuda, F. Nanba, et al., Black soybean seed coat polyphenols prevent AAPH-induced oxidative DNA-damage in HepG2 cells, *J. Clin. Biochem. Nutr.* 60 (2) (2017) 108–114, <https://doi.org/10.3164/jcbn.16-48>.
- [33] K. Soumya, K. Haridas, J. James, V. Sameer Kumar, L. Edatt, S. Sudheesh, Study of in vitro antioxidant and DNA damage protection activity of a novel luteolin derivative isolated from *Terminalia chebula*, *J. Taibah. Univ. Sci.* 13 (1) (2019) 755–763, <https://doi.org/10.1080/16583655.2019.1630892>.
- [34] L. Zhu, J. Chen, J. Tan, X. Liu, B. Wang, Flavonoids from *agrimonia pilosa ledeb*: free radical scavenging and DNA oxidative damage protection activities and analysis of Bioactivity-structure relationship based on molecular and electronic structures, *Molecules* 22 (3) (2017) 195, <https://doi.org/10.3390/molecules22030195>.
- [35] J.C. Wallet, V. Cody, A. Wojtczak, R.H. Blessing, Structural and conformational studies on bio-active flavonoids. Crystal and molecular structure of a complex formed between 2',6'-dimethoxyflavone and orthophosphoric acid: a model for flavone-nucleotide interactions, *Anti Cancer Drug Des.* 8 (5) (1993) 325–332.
- [36] A.D. Sarma, R. Sharma, Anthocyanin-DNA copigmentation complex: mutual protection against oxidative damage (1999). *Phytochemistry*, 52(7) 1313-1318.
- [37] E. Küpeli Akkol, Y. Genç, B. Karpuz, E. Sobarzo-Sánchez, R. Capasso, Coumarins and coumarin-related compounds in pharmacotherapy of cancer, *Cancers* 12 (7) (2020) 1959, <https://doi.org/10.3390/cancers12071959>.
- [38] P. Tiwari, Mishra, Role of flavonoids in DNA damage and carcinogenesis prevention, 08(04), *J. Carcinog. Mutagen.* (2017), <https://doi.org/10.4172/2157-2518.1000297>.
- [39] L. Leong, G. Shui, An investigation of antioxidant capacity of fruits in Singapore markets, *Food Chem.* 76 (1) (2002) 69–75, [https://doi.org/10.1016/s0308-8146\(01\)00251-5](https://doi.org/10.1016/s0308-8146(01)00251-5).
- [40] M. Ozaslan, I.D. Karagoz, I.H. Kilic, M.E. Guldur, Ehrlich ascites carcinoma, *Afr. J. Biotechnol.* 10 (13) (2011) 2375–2378.
- [41] R. Wong, Apoptosis in cancer: from pathogenesis to treatment, *J. Exp. Clin. Cancer Res.* 30 (1) (2011), <https://doi.org/10.1186/1756-9966-30-87>.
- [42] M. Greenwell, P.K. Rahman, Medicinal plants: their use in anticancer treatment, *Int. J. Pharmaceut. Sci. Res.* 6 (10) (2015) 4103–4112, [https://doi.org/10.13040/IJPSR.0975-8232.6\(10\).4103-12](https://doi.org/10.13040/IJPSR.0975-8232.6(10).4103-12).
- [43] O. Goni, M. Khan, M. Rahman, M. Hasan, F. Kader, N. Sazzad, et al., Pharmacological insights on the antidepressant, anxiolytic and aphrodisiac potentials of *Aglaonema hookerianum* Schott, *J. Ethnopharmacol.* 268 (2021) 113664, <https://doi.org/10.1016/j.jep.2020.113664>.
- [44] E. Küpeli Akkol, I. Tatlı Çankaya, G. Şeker Karatoprak, E. Carpar, E. Sobarzo-Sánchez, R. Capasso, Natural compounds as medical strategies in the prevention and treatment of psychiatric disorders seen in neurological diseases, *Front. Pharmacol.* 12 (2021), <https://doi.org/10.3389/fphar.2021.669638>.