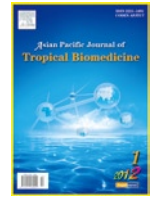




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Asian Pacific Journal of Tropical Biomedicine

journal homepage: [www.elsevier.com/locate/apjtb](http://www.elsevier.com/locate/apjtb)



Document heading

# Amelioration of oxidative stress induced by oxidative mutagens and COX–2 inhibitory activity of umbelliferone isolated from *Glycyrrhiza glabra* L

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## ARTICLE INFO

### Article history:

Received 13 January 2012

Received in revised form 22 January 2012

Accepted 15 April 2012

Available online 28 April 2012

### Keywords:

*Glycyrrhiza glabra* L

SOS chromotest

Comet assay

*Escherichia coli* PQ37

Human lymphocytes

COX–2 inhibition

## ABSTRACT

**Objective:** To evaluate antigenotoxic, antioxidant and COX–2 inhibitory potential of ‘GG–1’ fraction isolated by Medium Pressure Liquid Chromatography (MPLC) from ethyl acetate extract of *Glycyrrhiza glabra* (*G. glabra*) L rhizomes. **Methods:** The antigenotoxic activity was tested in human blood lymphocytes using comet assay and SOS chromotest using PQ37 strain of *Escherichia coli* (*E. coli*) against the diagnostic mutagens *viz.* 4–NQO and H<sub>2</sub>O<sub>2</sub>. Antioxidant activity was determined by DPPH radical scavenging assay. It was also investigated for the COX–2 inhibitory activity using Cayman COX (ovine) inhibitory screening assay. **Results:** The spectroscopic data of ‘GG–1’ fraction revealed it to be 7–hydroxycoumarin (Umbelliferone). In SOS chromotest, at the concentration of 616.75  $\mu$  M, umbelliferone exhibited moderate response by reducing the induction factor of hydrogen peroxide by 68.99% (IC<sub>50</sub> 223.44  $\mu$  M) and that of 4NQO by 59.71% (IC<sub>50</sub> 280.74  $\mu$  M). In comet assay, exhibited a good activity by inhibiting the genotoxicity of both hydrogen peroxide and 4NQO by 61.64% (IC<sub>50</sub> 330.02  $\mu$  M) and 50.66% (IC<sub>50</sub> 577.83  $\mu$  M) respectively. The umbelliferone exhibited free radical scavenging potential of 43.88% at 616.75  $\mu$  M. Umbelliferone possessed 95.68% inhibition of COX–2 at 10  $\mu$  M concentration with IC<sub>50</sub><1. **Conclusions:** The antigenotoxic, antioxidant and COX–2 inhibitory properties showed by the umbelliferone suggests that it may have several applications in nutraceuticals and human health care.

## 1. Introduction

Cancer is a multi–step process typically involving initiation, promotion and progression. Cancer is a major killer after heart disease and approximately all types of cancer are incurable at the time of diagnosis. This is second most common cause of death among children between the ages of 1 and 14 years in the United States[1]. Among the developed countries, cancer is the major health trouble and is becoming the major root cause of death in developing countries. The success has been achieved in curing non–metastatic cancer but most of the metastatic cancer cases

are incurable with conventional treatment methods such as surgery, radiotherapy and chemotherapy[2].

Over the last few years, numerous attempts have been tried to treat cancer and reduce the death rate caused by it. The statistics revealed that the occurrence and death rates of this dreadful disease cancer have not diminished[3]. Therefore, vital strategy against cancer is its prevention. Although extensive research in carcinogenesis has been done but advances in our understanding at the cellular and molecular levels of carcinogenesis have led to the development of a new promising approach for cancer prevention, known as chemoprevention. Cancer chemoprevention is the use of specific agents to inhibit, delay, or reverse the process of carcinogenesis. Since exposures to environmental carcinogens from diverse sources are unavoidable and it is important to find a way to counteract these carcinogens or protect cells from deleterious effects exerted by them[4]. Therefore, cancer chemoprevention offers a new practical approach to lessen the incidence of human cancer. Thus,

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Foundation No: DST: SR/FTP/LS–A–03/2001

in order to practice chemoprevention, it is essential to understand the mechanisms concerned with carcinogenesis. Numerous plants have been used in treatment of number of ailments on the basis of knowledge accumulated over centuries. Scientific research in this field has revealed that some of substances present in these medicinally important plants to be potentially toxic and carcinogenic. So, scientific exploration of traditionally used medicinal plants is necessary at two levels: as a source of possible chemotherapeutic agents and as a measure of safety for the continued use of medicinal plants in traditional medicines. The mutagenicity/carcinogenicity modulation by food constituents can remarkably change the concluding effects of genotoxins. The epidemiological evidence supports that high intake of dietary phytoconstituents effectively reduces the incidence of human cancer cases but particularly of epithelial cancers related to alimentary and respiratory tracts<sup>[5,6]</sup>. Cyclooxygenase (COX-2) plays a vital role in mediating the inflammatory process. COX-1 is constitutive isoenzyme that regulates homeostasis by maintaining the physiological level of prostaglandins. COX-2 is inducible and up-regulated by a number of stimuli such as cytokines, mitogens, oncogenes, growth factor and tumor promoters. Extra production of PGE<sub>2</sub> and increased COX-2 activity are oftenly observed in a variety of malignancies including breast, prostate, bladder, liver, pancreas, skin, lung, colon and brain. Therefore, prostaglandin synthesis suppression through the selective inhibition of COX-2 is now regarded as a new practical approach to cancer prevention<sup>[8,9]</sup>. Hence, it is not surprising that in present era much attention has been focused on antigenotoxic/antioxidant effects and COX-2 inhibitory activity of natural phytochemicals from plants.

*Glycyrrhiza glabra* (*G. glabra*) L. is an Ayurvedic medicinal plant. The rhizomes of this plant are used in number of traditional formulation to cure several diseases. There are reports regarding the bioactivities of *G. glabra*<sup>[10,11]</sup>. The present study deals with the isolation and characterization of a phytochemical from *G. glabra* L. The isolated fraction was tested for its antigenotoxic potential against genotoxins, hydrogen peroxide and 4-nitroquinoline-1-oxide in *Escherichia coli* (*E. coli*) PQ37 using SOS chromotest and in human blood lymphocytes using Comet assay. Earlier we reported many synthetic compounds as COX-2 inhibitors<sup>[12,13]</sup>. But as we know that natural products are safer for consumption in comparison to synthetic compounds, so in search of lead molecules from natural origin, we isolated a phytochemical and evaluated for its bioactive potential.

## 2. Materials and methods

### 2.1. Bacterial strains and chemicals

*E. coli* PQ37 strain was purchased from Institut Pasteur, France. Normal melting point agarose (NMPA), Low melting point agarose (LMPA), ethidium bromide, DPPH and

Ortho-nitrophenyl-β-D-galactopyranoside (ONPG), were purchased from Himedia Laboratories Pvt. Ltd., Mumbai, India. Para-nitrophenylphosphate (PNPP), was procured from Sisco Research Laboratories Pvt. Ltd., Mumbai, India; Polyethyleneglycol-4-tetraoctylphenoether (Triton X-100), hydrogen peroxide, dimethyl sulphoxide from Qualigens Fine Chemicals, Mumbai, India. Histopaque 1077 was purchased from Sigma Chemicals (St Louis, MO, USA). All other chemicals used were of analytical grade.

### 2.2. Plant material and isolation

The rhizomes of *G. glabra* L. were purchased from local market at Amritsar, Punjab, India. A voucher specimen no. 0342-B-03/2006, has been submitted to the Herbarium of Department of Botanical and Environmental Sciences, Guru Nanak Dev University, Amritsar, Punjab. The rhizomes (1 kg) were washed with running water to remove any dust impurities and dried at 40 °C. They were finely powdered and percolated with 80% methanol (3×5 litres) to obtain the methanol extract. It was made aqueous with distilled water in a separating funnel and further fractionated with series of organic solvents to obtain the fractions, viz. hexane fraction, chloroform fraction and ethyl acetate fraction. The resulting ethyl acetate extract was subjected to Medium Pressure Liquid Chromatography (MPLC), at a flow rate of 10 mL/min and 30 mBar pressure using Borosilikat 3.3, Code No. 17982 (Buchi Switzerland) column, packed with silica gel for flash chromatography (230-400 mesh) which led to the isolation of a white crystalline compound named as 'GG-1' from the 70:30 Hexane/ethyl acetate fraction of the column (Figure 1).

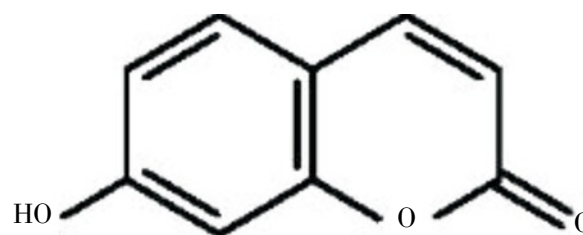


Figure 1. Structure of 7-hydroxycoumarin (Umbelliferone) M.wt-162.14.

### 2.3. Phytochemical analysis

The thin layer chromatography (TLC) of the fraction was performed on precoated Kieselgel 60F<sub>254</sub> plates (Merck, Germany) which revealed it to be a single compound named as GG-1 and was characterized structurally using NMR spectroscopic techniques.

### 2.4. Antigenotoxic activity

#### 2.4.1. SOS chromotest

For the SOS chromotest, an overnight culture of *E. coli* PQ37 (100 μL) was added to 5 mL of fresh La medium and incubated for 2 h at 37 °C. One ml of this culture was diluted

with 9 mL of L medium<sup>[14]</sup>. Aliquots of 600  $\mu$ L of bacterial suspension were distributed to series of glass test tubes, each containing 20  $\mu$ L of genotoxicant [ $\text{H}_2\text{O}_2$  (1mM)/4NQO (20  $\mu$ g/mL)] and 'GG-1' of different concentrations (19.2–616.75  $\mu$ M). Positive control was prepared by exposure of bacteria to either hydrogen peroxide or 4NQO alone. After incubation of 2 h at 37  $^\circ\text{C}$ , 300  $\mu$ L of the sample was used for assay of  $\beta$ -galactosidase and alkaline phosphatase activities respectively. The activity of the constitutive enzyme alkaline phosphatase was used as a measure of protein synthesis and toxicity. In order to determine the  $\beta$ -galactosidase activity, 2.7 mL of B-buffer (adjusted to pH 7.5) was added and after 10 min, 600  $\mu$ L of 0.4% 4-nitrophenyl- $\beta$ -galactopyranoside (ONPG) solution was added to each of the test tubes of one set. To determine the constitutive alkaline phosphatase activity, P-buffer (adjusted to pH 8.8) was added and after 10 min, 600  $\mu$ L of 0.4% 4-nitrophenyl phosphate (PNPP) solution was added to another set of tubes. All mixtures were incubated at 37  $^\circ\text{C}$  and observed for the colour development. After 30 min, the conversion of ONPG was stopped with 2 mL of 1 M sodium carbonate and that of PNPP with 2 mL of 1.5 N sodium hydroxide. The mixtures were centrifuged individually and absorption was measured at 420 nm using a reference solution in which culture was replaced by L medium.

The enzyme activities were calculated as:

$$\text{Enzyme units (U)} = A_{420} \times 1000/t$$

( $A_{420}$  = optical density at 420 nm; t = substrate conversion time in minutes).

$$\text{Induction factor (IF)} = R_c/R_0$$

$R_c$  =  $\beta$ -galactosidase activity/alkaline phosphatase activity determined for the test compound at concentration c

$R_0$  =  $\beta$ -galactosidase activity/alkaline phosphatase activity in the absence of the test compound.

Anti-genotoxicity was expressed as percentage inhibition of genotoxicity according to the formula:

$$\text{Inhibition (\%)} = 100 - (\text{IF}_1 - \text{IF}_0)/(\text{IF}_2 - \text{IF}_0) \times 100$$

where:

$\text{IF}_1$  is the induction factor of the 'GG-1'

$\text{IF}_2$  is the induction factor of positive control ( $\text{H}_2\text{O}_2/4\text{NQO}$ )

$\text{IF}_0$  the induction factor of the blank (without any test compound).

#### 2.4.2. Comet assay

The alkaline comet assay was performed on human blood lymphocytes<sup>[15]</sup>. Heparinized blood samples were obtained by venipuncture from a non-smoking, healthy male donor aged 25–40 years. Lymphocytes were isolated<sup>[16]</sup> and the viability of lymphocytes was determined by trypan blue exclusion test<sup>[17]</sup>.

Human peripheral blood lymphocytes ( $2 \times 10^6$  cells/mL) suspended in 1 mL phosphate buffer saline (PBS), were incubated for 30 min at 37  $^\circ\text{C}$  in BOD incubator with 20  $\mu$ L of hydrogen peroxide (25  $\mu$ M)/4NQO (5  $\mu$ g/mL) in the presence of different concentrations (19.20–616.75  $\mu$ M) of 'GG-1'. Each test compound/genotoxicant combination was tested thrice

in each experiment along with positive controls.

To evaluate the extent of DNA damage, images of 100 randomly selected cells stained with ethidium bromide, were analysed from each sample using an Epifluorescent Nikon microscope connected with a digital camera. Imaging was performed using a computerized image analysis system (Lucia Comet Assay Software 4.8 of Laboratory Imaging Ltd., UK) which acquires images, computes the integrated intensity profile for each cell, estimates the comet cell components (head and tail) and evaluates a range of derived parameters. Different concentrations of 'GG-1' were tested without hydrogen peroxide/4NQO (Negative Control).

Antigenotoxic activity of 'GG-1' was determined as

$$\text{Inhibition (\%)} = (T_1 - T_c)/(T_1 - T_0) \times 100$$

where:

$T_1$  = Tail moment induced by  $\text{H}_2\text{O}_2/4\text{NQO}$  (positive control)

$T_c$  = Tail moment of 'GG-1' in presence of  $\text{H}_2\text{O}_2/4\text{NQO}$

$T_0$  = Tail moment of negative control

#### 2.5. Free radical scavenging assay

'GG-1' fraction was tested for free radical scavenging activity<sup>[18]</sup>. About 300  $\mu$ L of the fraction (123.5–616.75  $\mu$ M) were added in 2 mL of DPPH (0.1 mM in methanolic solution). The reaction mixture was shaken well, placed at room temperature for 15 min and absorbance of the resulting solution was measured using spectrophotometer at 517 nm (Systronic 2202 UV-VIS spectrophotometer). The L-ascorbic acid and BHT were used as the standard antioxidants.

$$\text{Radical scavenging activity \%} = [(A_0 - A_1)/A_0 \times 100]$$

where

$A_0$  is the absorbance of DPPH solution.

$A_1$  is the absorbance of reaction mixture (with test sample).

#### 2.6. COX-2 inhibitory activity

*In vitro* COX-2 inhibiting activities of the 'GG-1' was evaluated using 'COX (ovine) inhibitor screening assay' kit with 96-well plates. Both ovine COX-1 and COX-2 enzymes were included. This screening assay directly measures  $\text{PGF}_{2\alpha}$  produced by  $\text{SnCl}_2$  reduction of COX-derived  $\text{PGH}_2$ . COX-1, COX-2, initial activity tubes were prepared taking 950  $\mu$ L of reaction buffer, 10  $\mu$ L of heme and 10  $\mu$ L of COX-1 and COX-2 enzymes in respective tubes. Similarly, COX-1, COX-2 inhibitor tubes were prepared by adding 20  $\mu$ L of inhibitor (GG-1) in each tube in addition to the above ingredients. The background tubes correspond to inactivated COX-1 and COX-2 enzymes obtained after keeping the tubes containing enzymes in boiling water for 3 min. along with vehicle control. Reactions were initiated by adding 10  $\mu$ L of arachidonic acid in each tube and quenched with 50  $\mu$ L of 1 M HCl.  $\text{PGH}_2$  thus formed was reduced to  $\text{PGF}_{2\alpha}$  by adding 100  $\mu$ L  $\text{SnCl}_2$ . The prostaglandin produced in each well was quantified using broadly specific prostaglandin antiserum that binds with major prostaglandins and reading the 96-well plate at 405 nm. The wells of the 96-well plate showing low absorption at 405 nm indicated the low level of

prostaglandins in these wells and hence the less activity of the enzyme. Therefore, the COX inhibitory activities of the compounds could be quantified from the absorption values of different wells of the 96-well plate.

### 2.7 Statistical analysis

The results are presented as the mean  $\pm$  standard deviation of three experiments. Regression analysis was carried out by best fit method to determine  $IC_{50}$  values. The significance of results was checked at  $P < 0.05$ .

## 3. Results

### 3.1. Structural elucidation of 'GG-1'

'GG-1' on thin layer chromatography showed  $R_f$  at 0.49 [solvent system: hexane (25): ethylacetate(10): acetic acid(1)]. Table 1 showed NMR values of GG-1. The  $^1H$ -NMR spectrum showed five signals in the aromatic region. Two these aromatic signals appeared as doublets at  $\delta$  6.72 (1H, d,  $J=8.4$  Hz) and 7.38 (1H, d,  $J=8.7$  Hz) were assignable at H-6 and H-5 respectively. Other two doublets at  $\delta$  6.11 (1H, d,  $J=9.3$  Hz) and 7.77 (1H, d,  $J=9.3$  Hz) were assigned to H-3 and H-4, respectively. One  $^1H$  signal appeared as singlet at  $\delta$  6.33 and could be assigned to H-8. Along with these aromatic signals, one  $D_2O$  exchangeable signal for OH functional group was also observed as a broad singlet at  $\delta$

4.54 (1H, br.s). The decoupled  $^{13}C$ -NMR spectrum revealed nine signals indicating presence of nine types of carbons. DEPT-90 spectrum shows five signals at  $\delta$  102.0, 110.9, 113.1, 129.2 and 144.6 and showing the presence of five CH carbons and absence of four signals in comparison to normal  $^{13}C$  spectrum shows the presence of four quaternary carbons. One signal at  $\delta$  162.3 was described to carbonyl function (C-2) of coumarin derivative. Another downfield intense signal at  $\delta$  161.7 was indicative of hydroxyl substitution at C-7 position. Therefore, on the basis of above evidences, the structure of 'GG-1' was established as 7-hydroxy coumarin (umbelliferone) (Table 1 & Figure 1).

**Table 1.**

$^1H$  NMR and  $^{13}C$  NMR spectral data of umbelliferone of *G. glabra* L.

Carbon No.	$^1H$ NMR	$^{13}C$ NMR
2	—	162.3
3	6.11	110.9
4	7.77 (d, 9.3 Hz)	144.6
4a	—	111.7
5	7.38 (d, 8.7 Hz)	129.2
6	6.72 (d, 8.4 Hz)	113.1
7	—	161.7
8	6.63 (s)	102.0
8a	—	155.0

### 3.2. Antigenotoxic assays

#### 3.2.1. SOS chromotest

**Table 2**

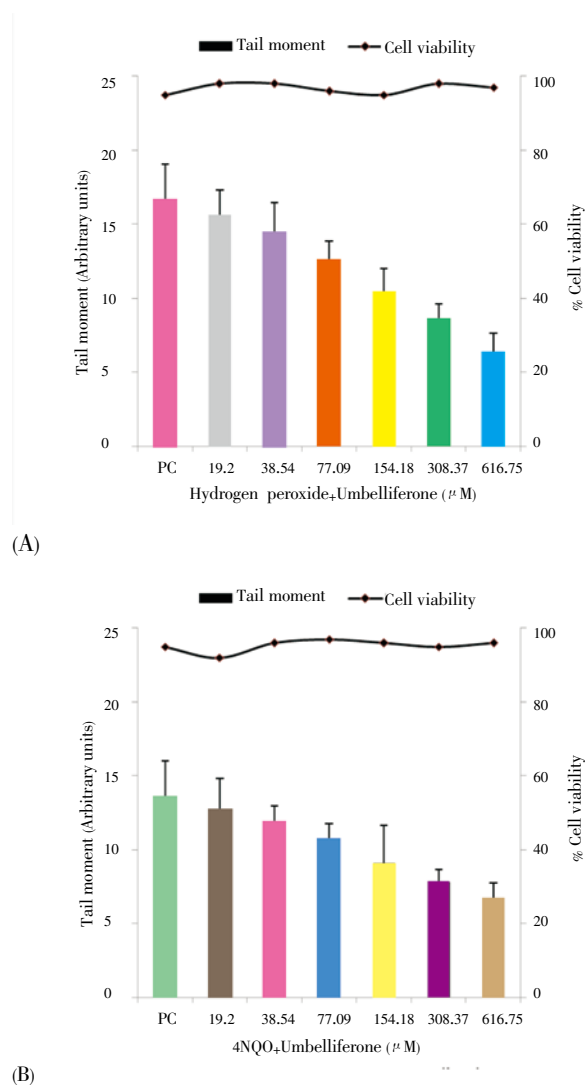
Effect of umbelliferone from *G. glabra* L. on genotoxicity of  $H_2O_2$  and 4NQO in SOS chromotest using *E. coli* PQ37 tester strain.

Treatment	Dose ( $\mu$ M)	$\beta$ -galactosidase units	Alkaline phosphatase units	Induction factor	Percent inhibition
Positive controls	1 mM	4.35 $\pm$ 0.08	13.10 $\pm$ 0.13	7.90	—
$H_2O_2$ 4NQO	20 $\mu$ g/mL	4.30 $\pm$ 0.06	13.10 $\pm$ 0.06	7.80	—
	0.00	0.55 $\pm$ 0.05	13.00 $\pm$ 0.05	1.00	—
	19.20	0.55 $\pm$ 0.05	12.98 $\pm$ 0.07	1.01	—
	38.54	0.60 $\pm$ 0.06	12.98 $\pm$ 0.12	1.10	—
	77.09	0.63 $\pm$ 0.02	13.01 $\pm$ 0.08	1.14	—
Negative control	154.18	0.58 $\pm$ 0.06	13.06 $\pm$ 0.09	1.05	—
	308.37	0.60 $\pm$ 0.04	13.05 $\pm$ 0.11	1.13	—
	616.75	0.59 $\pm$ 0.06	13.09 $\pm$ 0.12	1.07	—
	19.20	4.12 $\pm$ 0.12	13.00 $\pm$ 0.13	7.52	5.51
	38.54	3.80 $\pm$ 0.04	13.12 $\pm$ 0.10	6.88	14.79
$H_2O_2$ + GG-1	77.09	3.38 $\pm$ 0.10	13.10 $\pm$ 0.15	6.14	25.51
	154.18	2.70 $\pm$ 0.04	13.10 $\pm$ 0.09	4.90	43.48
	308.37	2.14 $\pm$ 0.02	13.13 $\pm$ 0.05	3.86	58.56
	616.75	1.73 $\pm$ 0.07	13.09 $\pm$ 0.07	3.14	68.99
	19.20	4.06 $\pm$ 0.09	12.97 $\pm$ 0.05	7.45	5.15
4NQO + GG-1	38.54	3.71 $\pm$ 0.05	13.00 $\pm$ 0.06	6.78	15.00
	77.09	3.12 $\pm$ 0.12	13.05 $\pm$ 0.10	5.69	31.03
	154.18	2.67 $\pm$ 0.11	13.05 $\pm$ 0.06	4.85	43.39
	308.37	2.17 $\pm$ 0.10	12.96 $\pm$ 0.09	3.97	56.33
	616.75	1.99 $\pm$ 0.15	12.60 $\pm$ 0.08	3.74	59.71

The antigenotoxic activity conferred by umbelliferone in SOS chromotest is shown in Table 2. At the concentration of 616.75  $\mu$ M, it exhibited moderate response by reducing the induction factor of hydrogen peroxide 68.99% ( $IC_{50}$  223.44  $\mu$ M) and that of 4NQO by 59.71% ( $IC_{50}$  280.74  $\mu$ M). The compound did not show any genotoxic activity itself (negative control). The standard butylated hydroxytoluene (BHT) showed  $IC_{50}$  value of 159.38  $\mu$ M and 159.83  $\mu$ M against  $H_2O_2$  and 4NQO respectively and ascorbic showed  $IC_{50}$  value of 18.33  $\mu$ M and 19.30  $\mu$ M against  $H_2O_2$  and 4NQO respectively.

### 3.2.2. Comet assay

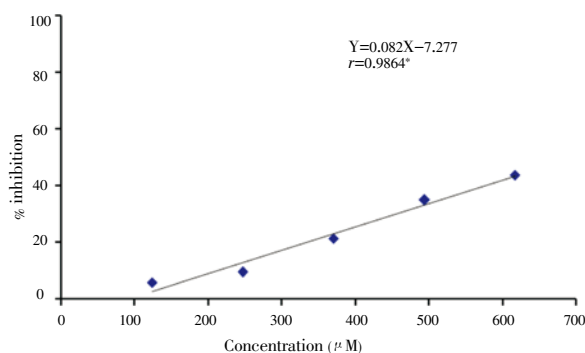
In comet assay, umbelliferone exhibited a good activity by inhibiting the genotoxicity of both hydrogen peroxide and 4NQO by 61.64% ( $IC_{50}$  330.02  $\mu$ M) and 50.66% ( $IC_{50}$  577.83  $\mu$ M) respectively (Figure 2). The standard BHT showed  $IC_{50}$  value of 140  $\mu$ M and 141.04  $\mu$ M against  $H_2O_2$  and 4NQO respectively and ascorbic showed  $IC_{50}$  value of 12.49  $\mu$ M and 14.70  $\mu$ M against  $H_2O_2$  and 4NQO respectively. Viability of the cells was observed to be greater than 90% at all the test doses, showing the non-cytotoxic nature of the compound.



**Figure 2.** Effect of 'GG-1' fraction from *G. glabra* on the tail moment induced by (A)  $H_2O_2$  (25  $\mu$ M) and (B) 4NQO (5  $\mu$ g/mL) in comet assay.

### 3.3. Antioxidant assay

The use of DPPH radical is very common method to determine the free radical scavenging activity. The umbelliferone exhibited free radical scavenging potential of 43.88% at 616.75  $\mu$ M. The result was compared with ascorbic acid and BHT (Figure 3).



**Figure 3.** Free radical scavenging effects of umbelliferone from *G. glabra* on DPPH radicals ( $^*P<0.05$ ).

### 3.4. COX-2 inhibitory assay

Umbelliferone possessed 95.68% inhibition of COX-2 at 10  $\mu$ M concentration with  $IC_{50} < 1$  and at the same concentration it inhibited the COX-1 by 56.91%. The standard drugs rofecoxib and celecoxib showed  $IC_{50}$  of 0.3 and 1.2  $\mu$ M[19].

## 4. Discussion

Medicinal plants are used traditionally as a potential source of chemotherapeutic drugs. Frequently used plants in traditional medicine are assumed to be safe due to their long term use and since they are natural, they considered having no side effects[20,21]. For centuries, medicinal plants have been used as remedies for human diseases because they contain phytoconstituents of therapeutic value. The use of plant extracts in food, cosmetics and pharmaceutical industries suggests that, in order to find active compounds, the study of medicinal plants is necessary[22,23]. The secondary metabolites from plants have antioxidative, antidiabetic, antimutagenic, anticarcinogenic, anti-inflammatory and cardioprotective effects, which owes to their free radical scavenging potential[24,25]. One of the potential uses of plant derived compounds is as antimutagenic agents and antioxidants. These phytochemicals may be useful in preventing cancer and mutation related diseases by fortifying physiological defense mechanisms or by acting as protective factors. Free radicals and other ROS are constantly generated *in vivo* and cause oxidative damage to biomolecules. This is regulated by existence of multiple antioxidants, DNA repair systems and replacement of damaged lipids and proteins.

ROS damage involves single or double stranded DNA breaks, purine and pyrimidine modifications, DNA intrastrand adducts and DNA crosslinks<sup>[26-29]</sup>. Increase in oxidative stress overwhelm repair systems and lead to cellular damage. Direct damage to DNA by ROS contribute to the development of cancer. Several evidences suggest a link between oxidative stress, cyclooxygenases and cancer<sup>[30,31]</sup>.

In the present study, 'GG-1' fraction was identified as umbelliferone, a coumarin fraction showed potent activity in modulating the genotoxicity of the oxidative mutagens. It modulated the genotoxicity of hydrogen peroxide (IC<sub>50</sub> 223.44  $\mu$ M) and that of 4NQO (IC<sub>50</sub> 280.74  $\mu$ M) in SOS chromotest by inhibiting the induction factor induced by these mutagens (Table 2). In comet assay, 'GG-1' fraction protected DNA from DNA damage as depicted by reduction of comet tail induced hydrogen peroxide (IC<sub>50</sub> 330.02  $\mu$ M) and 4NQO (IC<sub>50</sub> 577.83  $\mu$ M) respectively (Figure 2). The exhibited antigenotoxic activity of umbelliferone may be in part due to its radical scavenging activity which was checked by DPPH method. This is in consistence with reports in literature as paepalantine, an isocoumarin from *Paepalantes bromeliodes* (*P. bromeliodes*) has been reported to protect HepG2 cells from the oxidative stress induced by H<sub>2</sub>O<sub>2</sub><sup>[32]</sup>. Out of nine coumarins isolated from the n-butanol fraction of *Angelica gigas* Nakai, 3 compounds *viz.* marmesinin, nodakenin, columbianetin-O- $\beta$ -D-glucopyranoside showed potent neuroprotective activity<sup>[30]</sup>. Widleski *et al*<sup>[33]</sup> isolated five coumarin compounds (imperatorin, isoimperatorin, heraclenol, oxypeucedanin hydrate and heraclenin) from the fruits of *Angelica lucida* (*A. lucida*) and tested them for antibacterial activity. The GG-1 fraction exhibited moderate free radical scavenging potential of 43.88% at 616.75  $\mu$ M concentration (Figure 3). Torres *et al*<sup>[34]</sup> studied antioxidant activity of eight coumarins and two flavonols isolated from *Haplopappus multifolius* (*H. multifolius*) using the DPPH assay and reported that some of phenolic coumarins showed moderate antioxidant activity. Thoung *et al*<sup>[35]</sup> studied antioxidant phenolic coumarins and demonstrated that these compounds possess considerable antioxidant activities.

A series of 4-methyl-2H-chromen-2-one coumarin derivatives were evaluated for for *in vitro* cytotoxic activity against a human cancer cell line called Hep2 cells (Human Laryngeal cancer cells). Out of 10 compounds, screened for cytotoxicity, 7-(2-(4-fluorophenylamino)ethoxy)-4-methyl-2H-chromen-2-one, 7-(2-(2-hydroxyphenylamino)ethoxy)-4-methyl-2H-chromen-2-one and 7-(2-(2-methoxyphenylamino)ethoxy)-4-methyl-2H-chromen-2-one were found to be the most promising cytotoxic in this study<sup>[36]</sup>. Present investigation showed that GG-1 exhibits the potential to selectively inhibit COX-2 activity. This results show similarity to

literature reports on the COX-2 inhibitory activities of coumarin derivatives<sup>[37]</sup>. The molecule inhibited the COX-2 by 95.68% at 10<sup>-5</sup> M concentration. Results of our investigation are consistent with earlier reports that coumarin derivatives can decrease lipoxygenase, cyclooxygenase and iNOS expressions<sup>[38,39]</sup>. From the present study, it may be concluded that umbelliferone from *G. glabra* showed potent antigenotoxic/antioxidant and COX-2 inhibitory activity and need further detailed mechanistic studies as this molecule may serve as lead molecule for chemoprevention/chemotherapeutic studies.

### Conflict of interest statement

We declare that we have no conflict of interest.

### Acknowledgements

The authors are thankful to the Department of Science and Technology (DST No: SR/FTP/LS-A-03/2001), New Delhi (India) for funding the research project. The authors are also thankful to Dr. P.S. Ahuja, Director, IHBTP Palampur for providing laboratory facilities.

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