

## Biological Activities of the Natural Coumarins from Apiaceae Plants

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**Abstract:** Nineteen natural coumarins; badrakemin (1), colladonin (2), 14'-acetoxybadrakemin (3), anaticin (4), 14'-hydroxycolladonin (5), badrakemone (6), karatavicinol (7), 14'-acetoxybadrakemone (8), 14'-acetoxycolladonin (9), colladonin acetate (10), deltoin (11), smyrniodin (12), isoimperatorin (13), oxypeucedanin (14), bergapten (15), osthon (16), 4'-seneciolyxostol (17), neopapillarine (18), and scoparone (19) were tested against U87MG, A549, and PC3 cancer cell lines as well as against healthy human embryonic kidney cell line, HEK293. Colladonin (2) was found to have IC<sub>50</sub> values of 12.6 and 11.58 µM in A549 and PC3 cell lines, respectively. Deltoin (11) and 14'-acetoxybadrakemin (3) were found to have an IC<sub>50</sub> value of 9.92 µM and 11.85 µM against the U87MG cell line, respectively. Remarkably, these compounds show very low cytotoxicity against the healthy human embryonic kidney cell line, HEK293. In addition to the cytotoxic activity, nineteen natural coumarins were tested for their inhibitory activity against 5-LOX, collagenase, and elastase enzymes.

**Keywords:** Coumarin; cytotoxicity; collagenase; elastase; lipoxygenase. © 2023 ACG Publications. All rights reserved.

### 1. Introduction

Cancer remains one of the leading causes of death worldwide. While estimated deaths from the three leading cancer types for men are lung (67.160; 21%), prostate (34.700; 11%), and brain (11.020; 3%), the three leading cancer types for women are lung (59.910; 21%), breast (43.170; 15%), and brain

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(7.970; 3%), respectively, for 2023 in the United States [1]. Cancer is a disease with typical features such as abnormal cell growth, metastasis, invasion, and mutation accumulation potential [2]. In addition to these features, highly aggressive glioma is one of the primary tumors in the central nervous system [3]. Due to the invasive properties of gliomas, the incidence of which is increasing every year, the results of surgical interventions are not at the desired level because their boundary is unclear [4]. In addition, since chemotherapy treatment harms normal cells, it significantly reduces the quality of patient life. Therefore, it is still necessary to investigate new anti-tumor agents with specificity on tumor cells. Phytochemicals are of great therapeutic interest because they have minimal cytotoxicity to normal cells and are highly effective on some cancer cells [5].

Discovering novel drug candidates from natural sources against cancer has become a matter of great interest among researchers [6,7]. More than 1300 natural coumarin derivatives were reported from many plant species, fungi, and bacteria [8]. Coumarin derivatives have several biological and therapeutic properties such as antioxidant [9], antimicrobial [10], antiviral [11], antihyperlipidemic [12], antileishmanial [13], antitubercular [8], anti-inflammatory [9,14], anticancer [15-17] and butyrylcholinesterase inhibitory [18] properties. Coumarins have been reported to inhibit the growth of cancer cells, such as renal, lung, breast, and colon cancer cells [19-21]. So far, we have reported several natural coumarin derivatives with promising cytotoxic effect [22-24]. As part of our investigations on the biological activities of coumarins, 19 natural coumarin derivatives, namely badrakemin (**1**), colladonin (**2**), 14'-acetoxybadrakemin (**3**), anaticin (**4**), 14'-hydroxycolladonin (**5**) badrakemone (**6**), karatavicinol (**7**), 14'-acetoxybadrakemone (**8**), 14'-acetoxycolladonin (**9**), colladonin acetate (**10**), deltoin (**11**), smyrnioridin (**12**), isoimperatorin (**13**), oxypeucedanin (**14**), bergapten (**15**), osthol (**16**), 4'-seneciolyxostol (**17**), neopapillarine (**18**) and scoparone (**19**) (Figure 1), were examined for their cytotoxic and enzyme inhibitory activities.

## 2. Materials and Methods

### 2.1. General Experimental Procedures

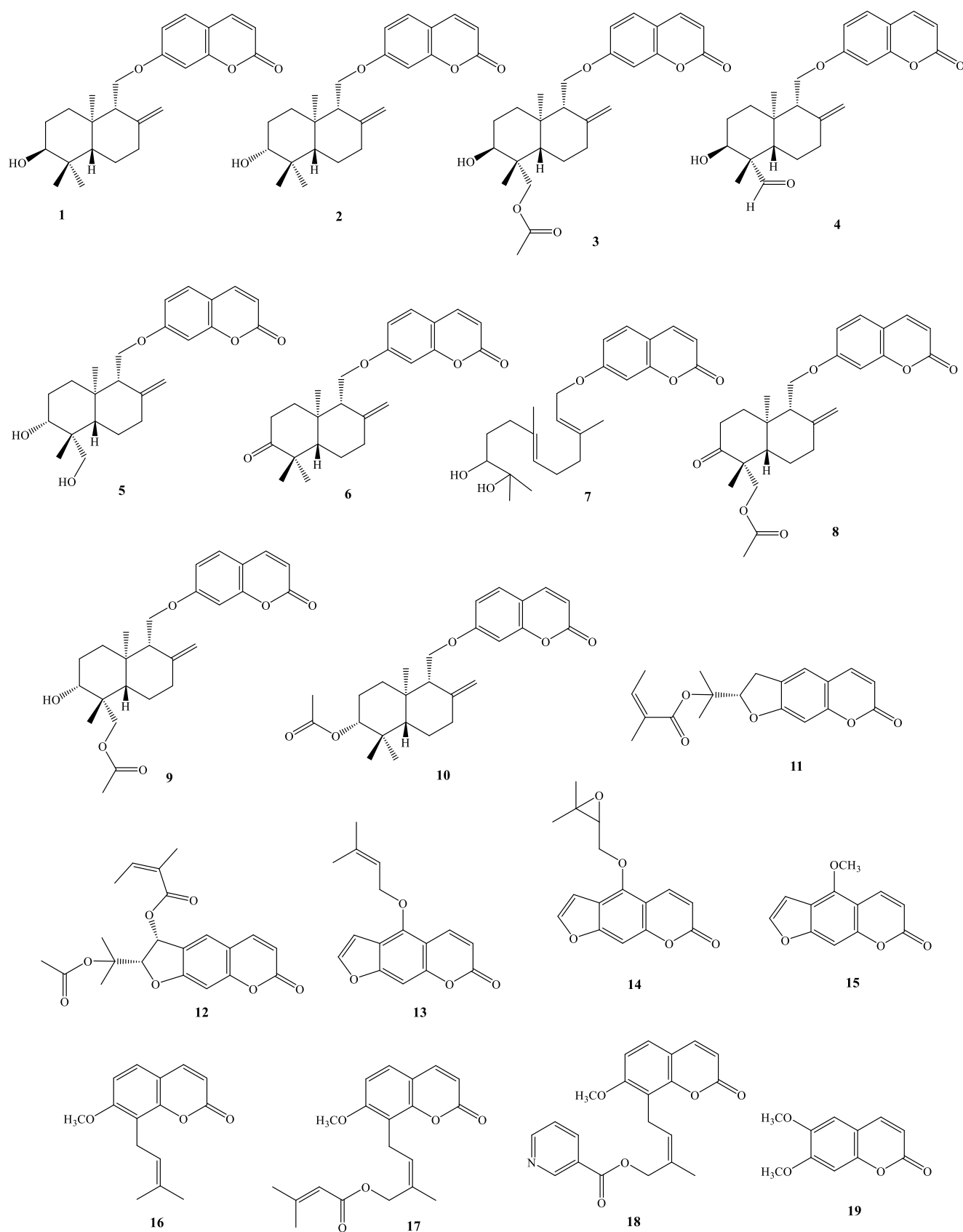
The MTT assay was measured using Spectramax i3 microplate reader (CA, USA). The absorbances were calculated with a microplate reader (BioTek, Winooski, USA). The reagents were purchased from Sigma and Gibco (CA, USA and Missouri, USA). Human glioma cancer cell line U87MG, human lung cancer cell line A549, human prostate cancer cell line PC3, and human embryonic kidney cell line HEK293 were provided from ATCC. Tris, SANA, NDGA, quercetin, and colchicine were obtained from Sigma (Missouri, USA).

### 2.2. Coumarin Derivatives

The coumarin derivatives used in this study were isolated from the roots of the following Apiaceae species: *Heptaptera anatolica* (Boiss.) Tutin; badrakemin (**1**), colladonin (**2**), 14'-acetoxybadrakemin (**3**), anaticin (**4**), 14'-hydroxycolladonin (**5**), badrakemone (**6**) and karatavicinol (**7**) [22]; *Heptaptera cilicica* (Boiss. & Balansa) Tutin; 14'-acetoxybadrakemone (**8**), 14'-acetoxycolladonin (**9**) and colladonin acetate (**10**) [23]; *Neocryptodiscus papillaris* (Boiss.) Herrnst. & Heyn; isoimperatorin (**13**), oxypeucedanin (**14**), bergapten (**15**), osthol (**16**), 4'-seneciolyxostol (**17**), neopapillarine (**18**), scoparone (**19**) [24]; *Petroedmondia syriaca* (Boiss.) Tamamsch; deltoin (**11**), and smyrnioridin (**12**) [25].

### 2.3. Cell Culture

All cell lines were cultured in DMEM supplemented with 10% heat-inactivated FBS, 1% (v/v) antibiotic-antimycotics solution and, 1% (v/v) non-essential amino acids at 37°C in 5% CO<sub>2</sub> [26].



**Figure 1.** Structures of the natural coumarins from Apiaceae plants used in the bioactivity testing

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### 2.3.1. Cytotoxicity Assay

Cytotoxic activities of coumarins were studied by cell proliferation analysis using the MTT assay [26]. Cells were seeded in 96-well plates of  $10^5$  cells per well and 24 h before treatment. The coumarins were dissolved in DMSO (less than 1%) and diluted with the medium. After incubation, the medium on the cells was removed, then washed with D-PBS. 30  $\mu$ L of MTT was added from the stock solution (5 mg/mL). At the end of the 4-hour incubation period, DMSO was added, and the culture plate was shaken at room temperature. The absorbance of the plate at a wavelength of 540 nm was measured in a microplate reader (Spectramax i3). Three experiments were performed.

### 2.4. Enzyme Inhibition Assays

#### 2.4.1. Elastase Inhibition Assay

In this assay, 2-amino-2-hydroxymethyl-propane-1,3-diol (Tris base), the porcine pancreatic elastase enzyme, and N-succinyl-Ala-Ala-Ala-p-nitroaniline (SANA) (substrate) were used to determine the elastase inhibitor activities of coumarins (**1-19**). Quercetin was used as a positive reference substance. The elastase enzyme, SANA, Tris base, and quercetin were obtained from Sigma, Inc. (MO, USA). The enzyme was dissolved in 0.2 mM Tris-HCl. SANA was dissolved in a 1.6 mM Tris-HCl buffer. 50  $\mu$ L of Tris-HCl buffer, 50  $\mu$ L solution of the coumarin compounds (at 20  $\mu$ g/mL concentration), and 25  $\mu$ L of the enzyme solution were pre-incubated (15 minutes) at room temperature. After incubation, 125  $\mu$ L of the substrate was added to initiate the reaction and incubated [27]. Enzyme inhibition of the coumarin compounds was measured at 410 nm.

#### 2.4.2. Collagenase Inhibition Assay

Collagenase inhibition was determined with a commercial assay kit (Colorimetric, Abcam 196999). The coumarin compounds (**1-19**) were first diluted in ethanol to 20  $\mu$ g/mL concentration for activity determination. The specified volume of compound solutions was added to the 96-well plate according to the instructions. Immediately after the substrate addition, the absorbance value in each plate well was measured using the BioTek SynergyHTX multi-mode plate reader. Enzyme inhibition of the coumarin compounds was measured at 345 nm (@ 37 °C) [27].

#### 2.4.3. Lipoxygenase (5-LOX) Inhibition Assay

The 5-LOX enzyme inhibition assay was performed by the UV spectrophotometric method described by Baylac and Racine [28]. The % inhibition was calculated as the absorbance changes at 234 nm for the 10 minutes of enzyme activity (at 25 °C) in the presence of linoleic acid and blank solvent or positive control standard (i.e., NDGA), which was then compared to the absorbance change for the 10 minutes of the enzyme activity (at 25 °C) in the presence of the tested coumarin solutions (at 20  $\mu$ g/mL concentration). NDGA was used as a standard. The experiments were performed in duplicates [29].

### 2.5. Statistical Analysis

All data were evaluated using GraphPad Prism® 7 program (La Jolla, California, USA). The statistical analysis was performed with ANOVA followed by Dunnett's or Tukey post hoc tests.

## 3. Results and Discussion

### 3.1. Cytotoxicity Results of the Coumarins (**1-19**)

In continuation of our investigations of the natural coumarins to discover cytotoxic natural compounds as leads for the development of novel anticancer drug substances, three coumarin derivatives; colladonin (**2**), 14'-acetoxycolladonin (**9**), and deltoin (**11**) were identified with the highest cytotoxic activity against A549 (lung adenocarcinoma), U87MG (brain malignant glioma), and PC3 (prostate) cancer cell lines. The IC<sub>50</sub> values of colladonin, 14'-acetoxycolladonin, and deltoin against A549 cells were 12.6, 20.49, and 19.0  $\mu$ M, respectively. The IC<sub>50</sub> values of these three compounds

against U87MG cells were 16.36, 17.61, and 9.92  $\mu\text{M}$ , respectively. In addition,  $\text{IC}_{50}$  values against PC3 cells were found as 11.58, 19.11, and 19.94  $\mu\text{M}$ , respectively. While cytotoxicity of all three coumarins on these cancer cell lines were comparable to that of colchicine (i.e., positive reference standard), their cytotoxicity on the non-cancerous human embryonic kidney cell line, HEK293, was considerably less cytotoxic than that of colchicine. Detailed results of all tested compounds are listed in Table 1.

**Table 1.**  $\text{IC}_{50}$  values of 19 coumarins ( $\mu\text{M}$ )

Compound	$\text{IC}_{50}$ ( $\mu\text{M}$ )			
	A549	U87MG	PC3	HEK293
<b>1</b>	>150	72 $\pm$ 3.0	87 $\pm$ 5.0	>150
<b>2</b>	13 $\pm$ 5.3	16 $\pm$ 3.4	12 $\pm$ 3.5	123 $\pm$ 5.3
<b>3</b>	37 $\pm$ 3.5	12 $\pm$ 3.0	30 $\pm$ 2.1	>150
<b>4</b>	>150	>150	47 $\pm$ 2.0	>150
<b>5</b>	54 $\pm$ 3.6	64 $\pm$ 2.5	46 $\pm$ 5.1	92 $\pm$ 3.4
<b>6</b>	33 $\pm$ 4.8	48 $\pm$ 2.6	43 $\pm$ 3.0	>150
<b>7</b>	32 $\pm$ 2.5	56 $\pm$ 3.0	29 $\pm$ 2.0	>150
<b>8</b>	26 $\pm$ 2.1	45 $\pm$ 3.0	52 $\pm$ 3.1	>150
<b>9</b>	20 $\pm$ 2.0	18 $\pm$ 2.0	19 $\pm$ 2.0	99 $\pm$ 4.3
<b>10</b>	47 $\pm$ 2.7	37 $\pm$ 3.0	26 $\pm$ 2.4	88 $\pm$ 3.6
<b>11</b>	19 $\pm$ 2.3	10 $\pm$ 3.0	20 $\pm$ 2.8	>150
<b>12</b>	51 $\pm$ 2.6	45 $\pm$ 3.0	70 $\pm$ 2.5	82 $\pm$ 3.8
<b>13</b>	-*	>150	-	>150
<b>14</b>	-	90 $\pm$ 4.0	-	>150
<b>15</b>	-	52 $\pm$ 4.0	>150	>150
<b>16</b>	-	>150	-	>150
<b>17</b>	43 $\pm$ 2.7	36 $\pm$ 2.2	-	>150
<b>18</b>	64 $\pm$ 2.8	74 $\pm$ 5.6	52 $\pm$ 1.0	87 $\pm$ 3.7
<b>19</b>	-	>150	-	>150
<b>Colchicine**</b>	23 $\pm$ 2.5	9 $\pm$ 2.5	5 $\pm$ 1.9	49 $\pm$ 2.9

\*Previously reported results were omitted. \*\*Positive control standard.

Hitherto, badrakemin (**1**), colladonin (**2**), 14'-acetoxybadrakemin (**3**), anaticin (**4**), 14'-hydroxycolladonin (**5**), badrakemone (**6**), karatavicinol (**7**), 14'-acetoxybadrakemone (**8**), 14'-acetoxycolladonin (**9**), colladonin acetate (**10**), deltoin (**11**), smyrnioridin (**12**), and neopapillarine (**18**) have not been tested against A549, U87MG and PC3 cancer cell lines and 4'-seneciolyxosthol (**17**) against A549 and U87MG cell lines. Especially colladonin (**2**), 14'-acetoxycolladonin (**9**), and deltoin (**11**) were identified as potent anticancer compounds with remarkable selectivity. The cytotoxic activity of these compounds against the cancer cell lines was several folds (in some cases 10x or more) more than their cytotoxicity activity against the non-cancerous HEK293 cell line. In addition, badrakemone (**6**), karatavicinol (**7**), and 4'-seneciolyxosthol (**17**) also show selective moderate anticancer potential. Specific cytotoxic activity of some of these coumarin derivatives against different cancer cell lines has been previously reported [22-24].

Isoimperatorin (**13**), oxypeucedanin (**14**), bergapten (**15**), osthol (**16**), and scoparone (**19**) were previously tested against A549 and PC3 cancer cell lines. Bergapten (**15**) was reported to inhibit 79.1  $\pm$  2.8% of A549 cancer cells at 50  $\mu\text{M}$  concentration [30,31]. The cytotoxic activity of osthol (**16**), a simple prenylated coumarin, against the PC3 cancer cell line was discovered in 2006 [32]. The  $\text{IC}_{50}$  value of osthol (**16**) was 20.1  $\mu\text{M}$  [33] against the PC3 cancer cell line. In another study, the  $\text{IC}_{50}$  values of osthol were determined to be 14.5 and 24.8  $\mu\text{g}/\text{mL}$  against PC3 and A549 cancer cell lines, respectively [34]. The  $\text{ED}_{50}$  values of isoimperatorin (**13**) and oxypeucedanin (**14**) against the A549 cancer cell line were determined as 12.2 and 9.5  $\mu\text{g}/\text{mL}$ , respectively [35]. While the  $\text{IC}_{50}$  value of isoimperatorin against the PC3 cell line was calculated as 119.4  $\mu\text{M}$ , the  $\text{IC}_{50}$  value of oxypeucedanin

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for the same cell line was more than 300  $\mu\text{M}$  [33]. The  $\text{IC}_{50}$  values of scoparone (**19**), a simple coumarin, against A549 and PC3 cell lines, were 18.2 and 18.7  $\mu\text{g}/\text{mL}$ , respectively [36]. Also, the  $\text{IC}_{50}$  value of 4'-seneciolyxosthol (**17**) against the PC3 cell line was reported as 421  $\mu\text{g}/\text{mL}$  [37].

## 3.2. Enzyme Inhibition Results of the Coumarins (1-19)

Collagenase, elastase, and lipoxygenase enzyme inhibitions of 19 natural coumarin compounds isolated from Apiaceae plants were also investigated. The enzyme inhibitor activities were carried out at a concentration of 20  $\mu\text{g}/\text{mL}$ , and the % inhibition values of the substances are given in Table 2.

**Table 2.** % Enzyme inhibition results of coumarins 1-19

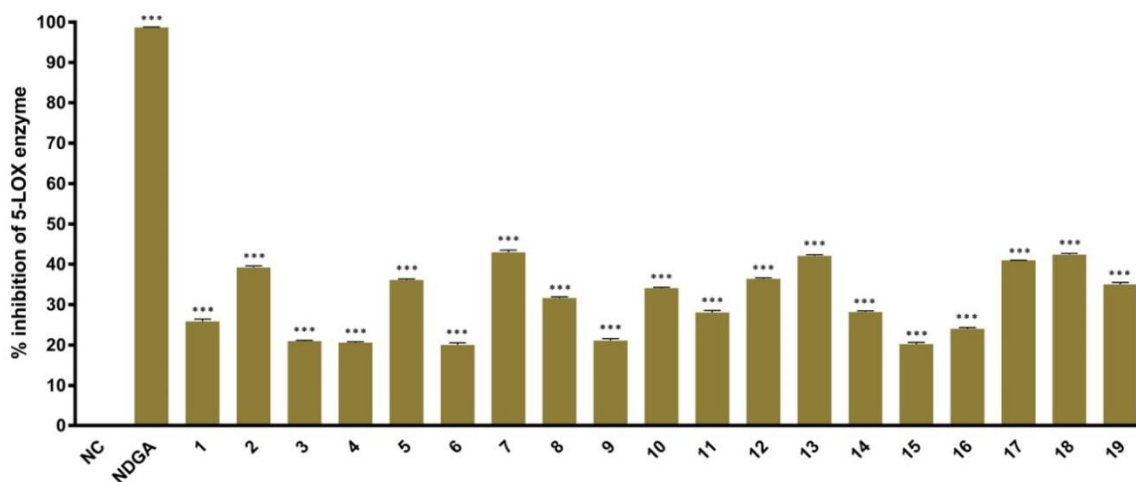
Compound	% Inhibition at 20 $\mu\text{g}/\text{mL}$ Concentration		
	Collagenase Enzyme	Elastase Enzyme	5-LOX Enzyme
<b>1</b>	10.1 $\pm$ 0.06	3.3 $\pm$ 0.04	25.3 $\pm$ 0.04
<b>2</b>	13.1 $\pm$ 0.11	16.7 $\pm$ 0.06	38.6 $\pm$ 0.05
<b>3</b>	3.2 $\pm$ 0.08	NE*	20.9 $\pm$ 0.11
<b>4</b>	NE	NE	20 $\pm$ 0.08
<b>5</b>	NE	NE	35.5 $\pm$ 0.09
<b>6</b>	NE	6.1 $\pm$ 0.04	19.5 $\pm$ 0.08
<b>7</b>	8 $\pm$ 0.07	18.2 $\pm$ 0.07	42.1 $\pm$ 0.01
<b>8</b>	13.6 $\pm$ 0.04	10.4 $\pm$ 0.08	31.3 $\pm$ 0.05
<b>9</b>	NE	4.8 $\pm$ 0.05	20.7 $\pm$ 0.06
<b>10</b>	8.6 $\pm$ 0.12	12.9 $\pm$ 0.08	33.7 $\pm$ 0.03
<b>11</b>	NE	9.8 $\pm$ 0.09	27.4 $\pm$ 0.03
<b>12</b>	4.3 $\pm$ 0.02	NE	36.2 $\pm$ 0.12
<b>13</b>	2.9 $\pm$ 0.01	22.2 $\pm$ 0.09	41.3 $\pm$ 0.09
<b>14</b>	12.2 $\pm$ 0.02	13.5 $\pm$ 0.01	27.8 $\pm$ 0.07
<b>15</b>	9.4 $\pm$ 0.1	7.1 $\pm$ 0.09	19.9 $\pm$ 0.09
<b>16</b>	4.7 $\pm$ 0.07	15.9 $\pm$ 0.07	23.6 $\pm$ 0.06
<b>17</b>	12.2 $\pm$ 0.02	10 $\pm$ 0.1	40 $\pm$ 0.03
<b>18</b>	6.2 $\pm$ 0.04	9.4 $\pm$ 0.07	41.6 $\pm$ 0.07
<b>19</b>	~**	NE	34.6 $\pm$ 0.09
<b>Quercetin***</b>	81.6 $\pm$ 0.06	74.9 $\pm$ 0.05	-
<b>NDGA***</b>	-	-	98.9 $\pm$ 0.07

SD values are shared as  $\pm$ . \*"Not Effective" at the tested concentration. \*\*Previously reported results were omitted. \*\*\*Positive control standards.

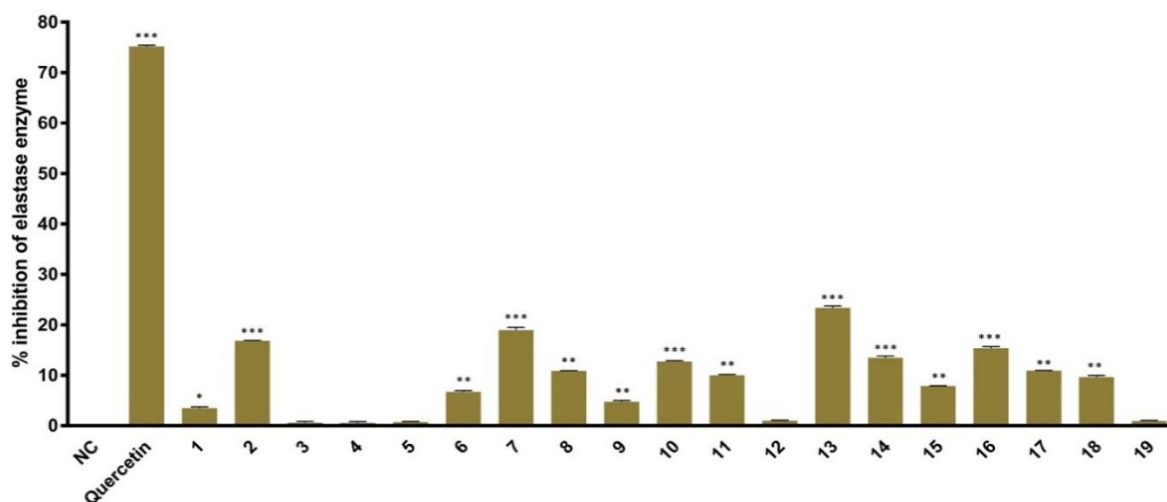
Karatavicinol (**7**), isoimperatorin (**13**), 4'-seneciolyxosthol (**17**), and neopapillarine (**18**) show about 40% inhibition of the 5-LOX enzyme at 20  $\mu\text{g}/\text{mL}$  concentration (Figure 2). This study demonstrated the anti-inflammatory effect of neopapillarine (**18**) for the first time. Isoimperatorin (**13**) was also found effective through different anti-inflammatory mechanisms in previous studies [38]. Karatavicinol (**7**) is another coumarin compound whose anti-inflammatory effect was demonstrated by *in vivo* methods [39]. The 5-LOX enzyme inhibition effect of bergapten (**15**) was investigated in earlier studies, but no effect was found [40]. In this study, 5-LOX enzyme inhibition of bergapten was also quite low compared to the other natural coumarin compounds. While the  $\text{IC}_{50}$  value of osthol (**16**) for the inhibition of 5-LOX enzyme was determined as 36.2  $\mu\text{M}$  in a previous study [41], in the present study, osthol exhibits 23.6% of the 5-LOX enzyme at 20  $\mu\text{g}/\text{mL}$  concentration. Due to the difference in the determination of inhibition results (i.e., % vs.  $\text{IC}_{50}$ ) of osthol in the current vs. previous study, a direct comparison of these results was not feasible. In a former LOX enzyme inhibition study of scoparone (**19**), a partial effect was detected as in the present study [42].

Isoimperatorin (**13**), karatavicinol (**7**), and colladonin (**2**) inhibited the elastase enzyme by 22.2, 18.2, and 16.7% at 20  $\mu\text{g}/\text{mL}$  concentration, respectively. These three compounds were the most effective coumarins against the elastase enzyme among the tested compounds. The elastase enzyme

inhibition value of the coumarins was between 15.9 - 3.3% at the tested concentration (Figure 3). Although these results were insignificant, to our knowledge, this study is the first study of the inhibition of elastase enzyme with these coumarins.



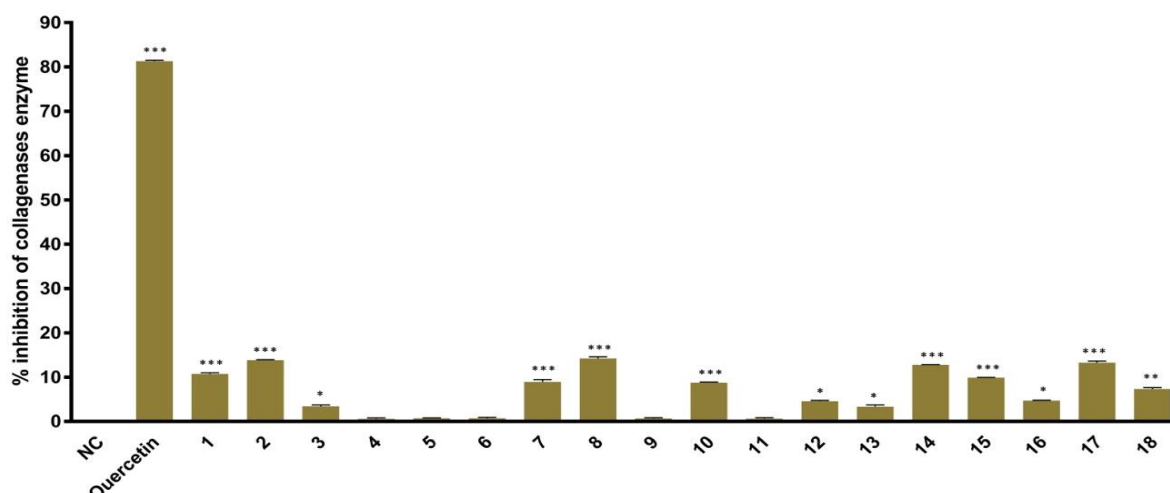
**Figure 2.** % LOX enzyme inhibition results of tested coumarins. NC: Negative control (Enzyme-free control group). NDGA (Nordihydroguaiaretic Acid): Positive control. The graph represents the mean  $\pm$  SD (n = 3). \*\*\*, p < 0.001



**Figure 3.** % Elastase enzyme inhibition results of tested coumarins. NC: Negative control (Enzyme-free control group). Quercetin: Positive control. The graph represents the mean  $\pm$  SD (n = 3). \*, p < 0.05; \*\*, p < 0.01; \*\*\*, p < 0.001

Except for scoparone (**19**) [43], 18 of these natural coumarins were tested for collagenase inhibitor activity. The collagenase enzyme inhibition activity of 14'-acetoxybadrakemone (**8**), colladonin (**2**), oxypeucedanin (**14**), and 4'-seneciolyxostol (**17**) was found to be 13.6, 13.1, 12.2, and 12.2%, respectively, at 20  $\mu$ g/mL concentration. The four coumarin compounds listed above are the most effective among the 18 coumarins; other compounds either did not inhibit the collagenase enzyme or had a very weak inhibition (Figure 4). Different coumarin compounds investigated in previous studies also showed weak activity against collagenase and elastase enzymes [44]. However, several examples of synthetic coumarins with strong inhibitor activity against these antiaging enzymes exist. Thus, perhaps semi-synthetic derivatives of natural coumarins may provide potential leads for antiaging compounds [45].

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**Figure 4.** % Collagenase enzyme inhibition results of tested coumarins. NC: Negative control(Enzyme-free control group). Quercetin: Positive control. The graph represents the mean  $\pm$  SD (n = 3). \*,  $p < 0.05$ ; \*\*,  $p < 0.01$ ; \*\*\*,  $p < 0.001$

19 different coumarin compounds were tested against 5-LOX, collagenase, and elastase enzymes. The 5-LOX enzyme inhibitions of the compounds were higher than the other investigated enzymes. The results of the current study are comparable to the previous 5-LOX, collagenase, and elastase enzyme inhibition studies that corroborate our findings.

#### 4. Conclusion

The cytotoxic activities of a series of plant-derived coumarin derivatives (**1-19**) on three cancer cell lines, A549, U87MG, and PC3, were investigated. Furthermore, the cytotoxic activity of these coumarins on non-cancerous human embryonic kidney cells, HEK293, was also evaluated. Colladonin (**2**), 14'-acetoxycolladonin (**9**), and deltoin (**11**), showed the strongest growth inhibition against A549, U87MG, and PC3 cell lines. The cytotoxicity of these coumarins against non-cancerous human embryonic kidney cells, HEK293, was several folds less than that of the positive control standard, colchicine. In addition, badrakemone (**6**), karatavicinol (**7**), and 4'-seneciolyxostol (**17**) were also demonstrated with moderate-level growth inhibition on the three cancer cell lines. Our cytotoxicity evaluations of the coumarin derivatives (**1-19**) finally put forward colladonin (**2**), 14'-acetoxycolladonin (**9**), and deltoin (**11**) as the most promising potential leads for designing novel drug candidates against lung, brain, and prostate cancers.

Elastase, collagenase, and 5-LOX enzyme inhibitions of 19 coumarin derivatives were also investigated. The % inhibition of coumarin derivatives at 20  $\mu\text{g/mL}$  concentration was evaluated and compared with the standards. Based on the results, the most active compounds for collagenase, elastase, and 5-LOX enzymes were 14'-acetoxybadrakemone (**8**) (13.6%), isoimperatorin (**13**) (22.2 %), and karatavicinol (**7**) (42.1%), respectively, even though the coumarin derivatives were not as effective as the positive controls. Nevertheless, using the active natural coumarin derivatives as the basic scaffold, various semi-synthetic coumarin derivatives can be prepared to design potent elastase, collagenase, and 5-LOX enzyme inhibitors.

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